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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Use of solube CD164 in inflammation and autoimmune disorders

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USE OF SOLUBLE CD164 IN INFLAMMATION AND AUTOIMMUNE DISORDERS (55)

FIELD OF THE INVENTION

The present invention relates to the field of inflammation and autoimmune disorders, in particular the discovery of a soluble glycosylated CD164 fragment useful for reducing inflammation and for treating inflammatory and/or autoimmune disorders.

BACKGROUND OF THE INVENTION

The following discussion is intended to facilitate the understanding of the invention, but is not intended nor admitted to be prior art to the invention.

CD164 is a member of the mucin-like receptor or sialomucin superfamily of glycoproteins. Sialomucins are transmembrane glycoproteins ranging from 50-3000 kD exhibiting limited similarity at the cDNA and amino acid levels. Mucin-like expressed proteins share the common characteristic of bearing numerous O-glycosylations linked to serine and threonine residues, which infer multiple kinds of cell-cell or cell-extracellular matrix interactions. The dense array of O-linked side chains are characterized by an extended structure that makes many of the mucin-like molecules long enough to protrude beyond the polysaccharide glycocalyx that surrounds the cell and also by the optimal exposure and high multiplicity of the terminal sugars. By virtue of the structural configuration as well as negative charge, mucin-like glycoproteins may act as a repulsive barrier unless a cell bear specific receptors for mucin (adhesion). Functions of mucin receptors depend on cell types and states of activation correlated with the core mucin peptide and with the cell-specific expression of glycosyl transferases, which in turn regulate the structure and presentation of the O-linked oligosaccharide sidechains, membrane anchorage, signal transduction abilities and or/the trafficking of the mucin to the correct cellular domain.

In its native state, CD164 is a disulphide-linked homodimer of two 80-85kDa subunits. CD64 is highly glycosylated, containing both O- and N-linked glycans. The extracellular region is comprised of two mucin domains (I and II) linked by a non-mucin domain containing intra-disulphide bridges as well as a cysteine-rich motif that resembles a consensus pattern previously found in growth factor and cytokine receptors. CD164 also contains a single-pass transmembrane domain and a 13-amino acid intracellular region that include a C-terminal motif (i.e. YHTL) able to target the protein to endosomes and lysosomes.

CD164 is an ortholog of murine MGC-24v (M. musculus) and rat (R.norvegicus) endolyn (endolyn-78), a membrane protein found in lysosomal and endosomal

compartment of mammalian cells. Other orthologs are found in B.Taurus, S.scrofa and chicken.

Four human CD164 mRNA species have been described arising by alternative splicing of six bona fide exons from a single genomic transcription unit located on human chromosome 6q21. There are probably 4 alternative promoters, two non-overlapping alternative last exons and one internal intron which is not always spliced out. The predominant CD164 (E1-6) isoform represents a 178 amino acid type I transmembrane glycoprotein. The other described isoforms are a sialomucin CD164 or CD164 isoform delta 5 containing 178 amino acids; a 184 residues CD164 isoform delta 4; and a 200 kD principally soluble isoform termed MGC-24 (for Multi-Glycosylated Core protein of 24 kD) lacking the transmembrane anchoring motif and having 189 residues. All isoforms are highly glycosylated proteins with O- and N-linked glycosylation sites.

CD164 functions include mediating, or regulating, haematopoietic progenitor cell adhesion and the negative regulation of their growth and/or differentiation. CD164 is usually expressed by CD34+ and $CD34^{lo/-}$ haematopoietic stem cells and associated microenvironmental cells. CD164 is also expressed by commited myeloid and erythroid colony forming cells, on bone marrow stromal and endothelial cells, weakly on lymphocytes, on mature CD20+ B, and on mesenchymal stem cells. This receptor may play a key role in haematopoisesis by facilitating the adhesion of human CD34+ cells to bone marrow stroma and by suppressing CD34+ $CD38^{lo/-}$ haematopoietic progenitor cell proliferation, acting as a potent signaling molecule. These effects involve the CD164 clas I and/or II epitopes recognized by the monoclonal antibodies (mAbs) 105A5 and 103B2/9E10. These epitopes are carbohydrate-dependent and are located on the N-terminal mucin domain I. The interaction of haemotopoietic cells with stromal/endothelial cells in their immediate microenvironment is thought to be of major importance in the regulation of haematopoietic stem self-renewal, quiescence, commitment and migration. These interactions involve cooperation between adhesion receptors, their cognate ligands and cytokines. A range of cell adhesion molecules (CAMS) including the Ig, integrin, cadherin, selectin and mucin-like families, participate in these processes. In an *in* vitro model, the cytoadherence of Plasmodium falciparum sexual stage parasites (gametocytes) to human bone marrow cells of stromal and endothelial origin was facilitated by CD164 presence. Developing stage III and IV gametocytes, but not mature stage V gametocytes, adhere to bone marrow cells.

In vitro, CD164 may also play a role in myogenic differentiation. Overexpression of CD164 in myoblast cell lines accelerated expression of biochemical markers of differentiation and enhanced formation of multinucleate myotubes, whereas antisense CD164 or soluble extracellular regions of CD164 inhibited myogenesis. Treatment of

myoblasts with sialidase or O-sialoglycoprotease, two enzymes that destroy CD164 functional epitopes, also inhibited differentiation.

The peanut agglutinin (PNA)-binding site of soluble MGC-24 represents a tumor associated carbohydrate marker expressed in many carcinomas. Total MGC-24 mRNA was found to be lower in human colorectal carcinomas as compared with normal adjacent mucosal tissues. Lymphatic vessel invasion by the carcinoma was correlated to low levels of MGC-24 mRNA in colon carcinomas, whereas high levels did correlate with less venous invasion and less remote metastasis.

Monoclonal antibodies specific for CD164 could prove useful for cancer diagnosis or therapy (EP889054, EP761814) and haematopoiesis inhibition (EP761814). The present invention is related to a new field of application for CD164 involving the treatment and/or prevention of inflammation and autoimmune disorders.

Although the primary function of the immune system is to protect an individual against infection by foreign invaders such as microorganisms, it may happen that the immune system attacks the individual's own tissues, leading to pathologic states known as autoimmune diseases, which are frequently associated with inflammatory processes. Examples of autoimmune diseases are rheumatoid arthritis, juvenile onset type I diabetes mellitus, systemic lupus erythematosus, thyroiditis and multiple sclerosis. Rheumatoid arthritis is a disease marked by signs and symptoms of inflammation of the joints. Systemic lupus erythematosus (SLE) is characterized by red, scaley patches on the skin and by malfunction of the kidneys at the advanced stage of the disease, and is associated with inflammatory reactions triggered by deposition of immune complexes in blood vessels, particularly in the kidneys. Multiple sclerosis is a human illness characterized by relapsing, inflammatory conditions that can cause weakness, body tremors and, in extreme cases, paralysis, and is associated with immune system attack of the protective myelin sheath surrounding peripheral nerve cells.

SUMMARY OF THE INVENTION

The present invention is based on the finding that CD164 has an inhibitory effect on various cytokines, namely interferon-γ, IL-2, IL-4, IL-5, IL-10 and TNF-α.

Therefore, It is a first object of the present invention to use a soluble CD164, or an isoform, mutein, fused protein, functional derivative, fragment, active fraction or salt thereof, for the manufacture of a medicament for treatment and/or prevention of inflammation and/or autoimmune disorders.

It is a second object of the present invention to use a soluble fragment of CD164 (herein designated as sf-CD164) as set forth by any of SEQ ID NO: 1, or SEQ ID NO: 2, or an isoform, mutein, fused protein, functional derivative, active fraction or salt thereof, for the manufacture of a medicament for treatment and/or prevention of inflammation and/or autoimmune disorders.

It is a third object of the present invention to provide for a pharmaceutical composition comprising a CD164, in the presence of one or more pharmaceutically acceptable excipients, suitable for reducing an inflammatory response, and/or for treatment and/or prevention of inflammation and/or autoimmune disorders.

It is a fourth object of the present invention to provide a method of inhibiting cytokine expression in an individual comprising administering to said individual a composition comprising a soluble CD164.

According to any of the objects of the present invention, preferably, said inflammation and/or autoimmune disease or disorder is particularly selected from the group consisting of chronic inflammatory disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents the amino acid alignment of soluble CD164 extracellular fragment (sf-CD164), delta 4-CD164, NP_006007 (Full length), MGC-24 and delta5-CD164. Signal peptide and extracellular domain are shown in dark gray and in light gray respectively.

"CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." Nucleic Acids Res 1994 Nov 11;22(22):4673-4680 Thompson JD, Higgins DG, Gibson TJ European Molecular Biology Laboratory, Heidelberg, Germany.

Figure 2 represents the SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de/) Domains alignment of soluble CD164 extracellular

fragment (sf-CD164), delta 4-CD164, NP_006007 (Full length), MGC-24 and delta5-CD164. For clarity purposes, the alignment is shown without the MGC-24 or CD164 domain represented (due to overlap).

Schultz et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 5857-5864

Letunic et al. (2002) *Nucleic Acids Res* 30, 242-244

Figure 3 represents the IFN-γ modulation by sf-CD164 *in vitro* administration. The X-axis represents the percentage of cytokine (IFN-γ) release and the Y-axis the sf-CD164 concentration in μg/ml.

Figure 4 represents the TNF-α modulation by sf-CD164 *in vitro* administration. The X-axis represents the percentage of cytokine (TNF-α) release and the Y-axis the sf-CD164 concentration in μg/ml.

Figure 5 represents the IL-2 modulation by sf-CD164 *in vitro* administration. The X-axis represents the percentage of cytokine (IL-2) release and the Y-axis the sf-CD164 concentration in μg/ml.

Figure 6 represents the IL-4 modulation by sf-CD164 *in vitro* administration. The X-axis represents the percentage of cytokine (IL-4) release and the Y-axis the sf-CD164 concentration in μg/ml.

Figure 7 represents the IL-5 modulation by sf-CD164 *in vitro* administration. The X-axis represents the percentage of cytokine (IL-5) release and the Y-axis the sf-CD164 concentration in μg/ml.

Figure 8 represents the IL-10 modulation by sf-CD164 *in vitro* administration. The X-axis represents the percentage of cytokine (IL-10) release and the Y-axis the sf-CD164 concentration in μg/ml.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, it has been found that the soluble glycosylated extracellular part of full length CD164 (herein designated as sf-CD164) has an inhibitory effect on various cytokines, namely interferon-γ, IL-2, IL-4, IL-5, IL-10 and TNF-α.

Therefore, It is a first object of the present invention to use a soluble CD164, or an isoform, mutein, fused protein, functional derivative, fragment, active fraction or salt thereof, for the manufacture of a medicament for treatment and/or prevention of inflammation and/or autoimmune disorders.

It is a second object of the present invention to use a soluble fragment of CD164 (herein designated as sf-CD164) as set forth by any of SEQ ID NO: 1, or SEQ ID NO: 2, or an isoform, mutein, fused protein, functional derivative, active fraction or salt thereof, for the manufacture of a medicament for treatment and/or prevention of inflammation and/or autoimmune disorders.

Preferabily, according to any of the preceding objects, the soluble CD164 is

(a) the polypeptide as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6;

(b) a polypeptide exhibiting at least about 85% identity to the polypeptide as set forth in any of SEQ ID NO:1, or SEQ ID NO: 6;

(c) a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO: 1, or SEQ ID NO: 6;

(d) a polypeptide comprising the extracellular part of an anchored CD164 (harboring a transmembrane domain);

(e) the mature form of the polypeptide whose sequence is recited in SEQ ID NO: 6;

(f) the histidine tag form of the polypeptides whose sequences are recited in SEQ ID NO: 1 (SEQ ID NO: 2) or SEQ ID NO: 6;

Preferabily, according to any of the preceding objects, the soluble CD164 is a glycosylated CD164.

Most preferabily, the glycosylated CD164 is glycosylated at any of the positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6.

The N-glycosylation sites (asparagine glycosylation) in sf-CD164 are located at residues 3, 9, 18, 49, 54, 71, 81, 98 and 123. The O-glycosylated sites in sf-CD164 are located at residues 11, 12, 17, 20, 21, 25, 26, 31, 32, 89, 90, 92, 96, 99, 100, 104, 108, 110, 111, 112, 113, 115, 117, 118, 119, 121, 122, 125, 127, 129, 130, 136.

The N-glycosylation sites (asparagine glycosylation) in MGC-24 (with signal peptide, SEQ ID NO: 6) are located at residues 26, 32, 41, 72, 77, 94, 104, 121 and 146. The O-glycosylated sites in MGC-24 (with signal peptide) are located at

residues 34, 35, 40, 43, 44, 48, 49, 54, 55, 112, 113, 115, 119, 122, 123, 127, 131, 133, 134, 135, 136, 138, 140, 141, 142, 144, 145, 148, 150, 152, 153, 159.

Still preferabily, according to any of the preceding objects, the soluble CD164 is a phosphorylated CD164.

Still most preferabily, the phosphorylated CD164 is phosphorylated at any of the positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6.

The phosphorylation sites in sf-CD164 are divided in:

(i) cAMP- and cGMP-dependent protein kinase phosphorylation sites located at residues 134 to 137;

(ii) protein kinase C phosphorylation sites located at residues 100 to 102 and 112 to 114;

(iii) casein kinase II phosphorylation sites located at residues 73 to 76 and 136 to 139.

Still preferabily, according to any of the preceding objects, the soluble CD164 is a myristoylated CD164.

The N-myristoylation site in sf-CD164 is located at residue 119.

Still most preferabily, the myristoylated CD164 is myristoylated at any of the positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6.

It is a third object of the present invention to provide for a pharmaceutical composition comprising a soluble CD164, in the presence of one or more pharmaceutically acceptable excipients, suitable for reducing an inflammatory response, and/or for treatment and/or prevention of inflammation and/or autoimmune disorders.

It is a fourth object of the present invention to provide a method of inhibiting cytokine expression in an individual comprising administering to said individual a composition comprising a soluble CD164.

According to the fourth object of the present invention, further preferred is a method of inhibiting TNF-alpha release comprising providing or administering to individuals in need thereof said pharmaceutical or physiologically acceptable composition described in the third object.

According to the fourth object of the present invention, further preferred is a method of inhibiting IFN-γ release comprising providing or administering to individuals

in need thereof said pharmaceutical or physiologically acceptable composition described in the third object.

According to the fourth object of the present invention, further preferred is a method of inhibiting IL-2 release comprising providing or administering to individuals in need thereof said pharmaceutical or physiologically acceptable composition described in the third object.

According to the fourth object of the present invention, further preferred is a method of inhibiting IL-4 release comprising providing or administering to individuals in need thereof said pharmaceutical or physiologically acceptable composition described in the third object.

According to the fourth object of the present invention, further preferred is a method of inhibiting IL-5 release comprising providing or administering to individuals in need thereof said pharmaceutical or physiologically acceptable composition described in the third object.

According to the fourth object of the present invention, further preferred is a method of inhibiting IL-10 release comprising providing or administering to individuals in need thereof said pharmaceutical or physiologically acceptable composition described in the third object.

According to any of the objects of the present invention, preferably, said inflammation and/or autoimmune disease or disorder is selected from the group consisting of chronic inflammatory disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis.

In preferred embodiments of the compositions of the invention disclosed herein, compositions of the invention may further comprise any combination of a soluble CD164 of any of the previous objects of the invention and a known medicament in the art that treats inflammation and/or autoimmune disorders such that the composition produces a biological effect greater than the expected effect for

said soluble CD164 administered alone rather than in combination with a known medicament in the art that treats inflammation and/or autoimmune disorders.

The following definitions are set forth to illustrate and define the meaning and scope of the terms used to describe the invention herein.

As used interchangeably herein, the terms "oligonucleotides", and "polynucleotides" and nucleic acid include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form. The terms encompass "modified nucleotides" which comprise at least one modification, including by way of example and not limitation: (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar. For examples of analogous linking groups, purines, pyrimidines, and sugars see for example PCT publication No. WO 95/04064. The CD164 polynucleotide sequences may be prepared by any known method, including synthetic, recombinant, *ex vivo* generation, or a combination thereof, as well as utilizing any purification methods known in the art.

The terms polynucleotide construct, recombinant polynucleotide and recombinant polypeptide are used herein consistently with their use in the art. The terms "upstream" and "downstream" are also used herein consistently with their use in the art. The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein and consistently with their use in the art. Similarly, the terms "complementary", "complement thereof", "complement", "complementary polynucleotide", "complementary nucleic acid" and "complementary nucleotide sequence" are used interchangeably herein and consistently with their use in the art.

The term "purified" is used herein to describe a CD164 polynucleotide or polynucleotide vector that has been separated from other compounds including, but not limited to, other nucleic acids, carbohydrates, lipids and proteins (such as the enzymes used in the synthesis of the polynucleotide). Purified can also refer to the separation of covalently closed polynucleotides from linear polynucleotides, or vice versa, for example. A polynucleotide is substantially pure when at least about 50%, 60%, 75%, or 90% of a sample contains a single polynucleotide sequence. In some cases this involves a determination between conformations (linear versus covalently closed). A substantially pure polynucleotide typically comprises about 50, 60, 70, 80, 90, 95, 99% weight/weight of a nucleic acid sample. Polynucleotide purity or homogeneity may be indicated by a number of means well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polynucleotide band upon staining the gel. For certain purposes, higher resolution can be achieved by using HPLC or other means well known in the art.

Similarly, the term "purified" is used herein to describe a CD164 polypeptide that has been separated from other compounds including, but not limited to, nucleic acids, lipids, carbohydrates and other proteins. In some preferred embodiments, a polypeptide is substantially pure when at least about 50%, 60%, 75%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% of the polypeptide molecules of a sample have a single amino acid sequence. In some preferred embodiments, a substantially pure polypeptide typically comprises about 50%, 60%, 70%, 80%, 90% 95%, 96%, 97%, 98%, 99% or 99.5% weight/weight of a protein sample. Polypeptide purity or homogeneity is indicated by a number of methods well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution can be achieved by using HPLC or other methods well known in the art.

Further, as used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Alternatively, purification may be expressed as "at least" a percent purity relative to heterologous polynucleotides (DNA, RNA or both) or polypeptides. As a preferred embodiment, the CD164 polynucleotides or polypeptides are at least; 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 96%, 98%, 99%, 99.5% or 100% pure relative to heterologous polynucleotides or polypeptides. As a further preferred embodiment the polynucleotides or polypeptides have an "at least" purity ranging from any number, to the thousandth position, between 90% and 100% (e.g., at least 99.995% pure) relative to heterologous polynucleotides or polypeptides. Additionally, purity of the polynucleotides or polypeptides may be expressed as a percentage (as described above) relative to all materials and compounds other than the carrier solution. Each number, to the thousandth position, may be claimed as individual species of purity.

The term "isolated" requires that the material be removed from its original environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

Specifically excluded from the definition of "isolated" are: naturally occurring chromosomes (*e.g.*, chromosome spreads), artificial chromosome libraries, genomic

libraries, and cDNA libraries that exist either as an *in vitro* nucleic acid preparation or as a transfected/transformed host cell preparation, wherein the host cells are either an *in vitro* heterogeneous preparation or plated as a heterogeneous population of single colonies. Also specifically excluded are the above libraries wherein a 5' EST makes up less than 5% (or alternatively 1%, 2%, 3%, 4%, 10%, 25%, 50%, 75%, or 90%, 95%, or 99%) of the number of nucleic acid inserts in the vector molecules. Further specifically excluded are whole cell genomic DNA or whole cell RNA preparations (including said whole cell preparations which are mechanically sheared or enzymatically digested). Further specifically excluded are the above whole cell preparations as either an *in vitro* preparation or as a heterogeneous mixture separated by electrophoresis (including blot transfers of the same) wherein the CD164 polynucleotide have not been further separated from the heterologous polynucleotides in the electrophoresis medium (*e.g.*, further separating by excising a single band from a heterogeneous band population in an agarose gel or nylon blot).

The term "primer" denotes a specific oligonucleotide sequence that is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by DNA polymerase, RNA polymerase, or reverse transcriptase.

The term "polypeptide" refers to a polymer of amino acids without regard to the length of the polymer. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not specify or exclude post-expression modifications of polypeptides. For example, polypeptides that include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups, myristoylated groups and the like are expressly encompassed by the term polypeptide. Also included within the definition are phosphorylated or dephosphorylated polypeptides. Also included within the definition are polypeptides which contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

Without being limited by theory, the compounds/polypeptides of the invention are capable of inhibiting proinflammatory- and/or immune-related cytokine expression, and are thus believed to treat "inflammation and/or autoimmune disorders".

Preferred "inflammation and/or autoimmune disorders" believed to involve excessive or dysregulated inflammation or immune response include chronic inflammatory disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis.

The term "heterologous", when used herein, is intended to designate any polypeptide or polynucleotide other than a CD164 polypeptide or a polynucleotide encoding a CD164 polypeptide of the present invention.

The terms "comprising", "consisting of" and "consisting essentially of" are defined according to their standard meaning. A defined meaning set forth in the M.P.E.P. controls over a defined meaning in the art and a defined meaning set forth in controlling Federal Circuit case law controls over a meaning set forth in the M.P.E.P. With this in mind, the terms may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

The term "CD164-related diseases and disorders" as used herein refers to any disease or disorder comprising an aberrant functioning of CD164, or which could be treated or prevented by modulating CD164 levels or activity. "Aberrant functioning of CD164" includes, but is not limited to, aberrant levels of expression of CD164 (either increased or decreased, but preferably decreased), aberrant activity of CD164 (either increased or decreased), and aberrant interactions with ligands or binding partners (either increased or decreased). By "aberrant" is meant a change from the type, or level of activity seen in normal cells, tissues, or patients, or seen previously in the cell, tissue, or patient prior to the onset of the illness. In preferred embodiments, these CD164-related diseases and disorders include inflammation and autoimmune diseases and disorders described previously.

The term "treating" as used herein refers to administering a compound after the onset of clinical symptoms.

The term "in need of treatment" as used herein refers to a judgment made by a caregiver (*e.g.* physician, nurse, nurse practitioner, etc in the case of humans; veterinarian in the case of animals, including non-human mammals) that an individual

or animal requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that include the knowledge that the individual or animal is ill, or will be ill, as the result of a condition that is treatable by the compounds of the invention.

The term "individual" or "patient" as used herein refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans. The term may specify male or female or both, or exclude male or female.

The term "non-human animal" refers to any non-human vertebrate, including birds and more usually mammals, preferably primates, animals such as swine, goats, sheep, donkeys, horses, cats, dogs, rabbits or rodents, more preferably rats or mice. Both the terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

The term "prevention" within the context of this invention refers not only to a complete prevention of the disease or one or more symptoms of the disease, but also to any partial or substantial prevention, attenuation, reduction, decrease or diminishing of the effect before or at early onset of disease.

The term "treatment" within the context of this invention refers to any beneficial effect on progression of disease, including attenuation, reduction, decrease or diminishing of the pathological development after onset of disease.

The term "CD164", as used herein, is intended to include human CD164, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its salts, functional derivatives, variants, analogs and active fragments. As such "CD164" also include all sequences as set forth in the sequence listing as SEQ ID NO: 1, or SEQ ID NO: 2, or SEQ ID NO: 3, or SEQ ID NO: 4, or SEQ ID NO: 5, or SEQ ID NO: 6. The use of CD164 of human origin is also preferred in accordance with the present invention. The term CD164, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and active fragments thereof.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent application, issued U.S. or foreign patents or any other references, are entirely incorporated by reference herein, including all data, tables, figures and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various application such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning an range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

The instant invention encompasses the use of a CD164 polypeptide as an important new tool to control inflammatory response or/and autoimmune disease. It has been found that a soluble CD164 significantly inhibits cytokines, of which IFN-γ, IL-5, IL-4, IL-2, IL10 and TNF-α.

CD4+T cells can be assigned to two different subsets called T helper type 1 cells (Th1) and T helper type 2 cells (Th2) on the basis of distinct, non-overlapping cytokine expression patterns. Th1 is characterized by the secretion of IL-2, interferon-γ, IL-12 and TNF-α, and Th2 by the secretion of IL-4, IL-5, IL-9, IL-10 and IL-13. Nevertheless, these are not strict subsets as IFN-γ and IL-10 can suppress effects associated with Th1 as well as Th2 responses, and IL-4 and IL-13 are also able to promote the production of IL-12, thereby promoting Th1 and potentially inhibiting Th2 responses. Hence, in selected cases, IFN-γ can oppose inflammation, whereas IL-4 can promote inflammation. TNF-α antagonists are currently used in studies for beneficial effect on cellular injury in rheumatic diseases (e.g. rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, spondylarthropathies), inflammatory bowel disease, endotoxemia and Crohn's disease. Studies with TNF-α inhibitors in other inflammatory conditions such as adult Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis have shown

promising results (Tutuncu, 2002). Th1 T cells are able to mediate macrophage activation and delayed-type hypersensitivity (DTH), giving rise to pro-inflammatory or cell-mediated immune responses, whereas Th2 T cells promote IgG1 and IgE secretion leading to immediate-type hypersensitivity reactions (humoral immunity; stimulate antibody-mediated responses, activate mast cells, and elicit tissue eosinophilia). Th1 is a key feature in the pathogenesis of diseases like rheumatoid arthritis, sarcoidosis, and tuberculosis, whereas Th2 is involved in allergy, antiparasite responses and in the asthmatic airway (e.g. role in fibrosis) where elevated levels of IL-4, IL-5, IL-13 and IL-9 have been detected (O Gor, 2003; Herrick, 2003).

Allergic inflammation is consistent with a Th2-cell-based aetiology of atopic disease. For example, defective priming of Th2 cells in the absence of IL-4 resulted in a failure to generate allergic inflammatory responses after subsequent airway challenge. IL-5 and IL-13 have been shown to be more directly responsible for the characteristic eosinophil infiltrates and mucus hypersecretion. IL-10 has also been shown to suppress airway inflammation.

In multiple sclerosis, Th1 mediated immune responses are thought to promote the disease, whereas Th2 mediated immune responses are believed to have an ameliorating effect on the progression of the disease. T cells expressing IL-10 have been shown to suppress experimental autoimmune encephalomyelitis (EAE), a rat model for multiple sclerosis. IL-4 has been reported to inhibit the down-modulating effect of IL-10 on inflammation. TNF-α has been hypothesized to be responsible for the induction of EAE (TNF-α can be secreted by both Th1 and Th2 cultures).

Human systemic lupus erythematosus (SLE) is considered to be driven by a Th2 response. However, IFN-γ has been shown to have a major effect on disease progression in a mouse model. IL-10 seems to be of importance for lupus amelioration (also in psoriasis, inflammatory bowel disease, chronic hepatitis C), whereas IL-4 is expected to mediate disease maintenance.

Myocarditis is defined by inflammation of the heart muscle and is thought to be mediated by an autoimmune response to a cardiac-specific antigen after an acute upper respiratory infection. The severity of the experimental autoimmune myocarditis (EAM) in the mouse model is reduced by administration of anti-IL-4, indicating a role of IL-4 in disease progression. In contrast, mice genetically deficient in IFN-γ

production, or with the use of IFN-γ antibodies, show exacerbation of EAM after immunization. IL-10 has been shown to reduce progression of EAM.

Therefore, the inventors believe that CD164 is involved in preventing excessive and prolonged inflammatory responses as well as acting on autoimmune diseases by modulating the immune response, and a CD164 polypeptide antagonizes the onset of inflammatory and/or autoimmune disorders, particularly multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis.

PREFERRED EMBODIMENTS OF THE INVENTION

I. CD164 Soluble Polypeptide of the Invention

By "intact" or "full-length" CD164 polypeptide as used herein is meant the full-length polypeptide sequence of CD164 having all six exons, from the N-terminal methionine to the C-terminal stop codon. Example of intact or full length CD164 polypeptide is found in the sequence listing as SEQ ID NO: 3. The term "CD164 soluble polypeptides" as used herein refers to soluble fragments of the "intact" or "full-length" CD164 polypeptide, to the soluble polypeptide as set forth in SEQ ID NO: 6 (MGC-24), as well as to the polypeptides as set forth in SEQ ID NO: 4 or SEQ ID NO: 5, that have "inflammation and/or immune-related activity". The term "fragment" means a polypeptide having a sequence that is entirely the same as part, but not all, of an intact or full-length CD164 polypeptide, of a soluble polypeptide as set forth in SEQ ID NO: 6 (e.g. sf-CD164 is a fragment), and of polypeptides as set forth in SEQ ID NO: 4 or SEQ ID NO: 5 as well as any other CD164 polypeptides whether soluble or non-soluble. Such fragments may be "free-standing" (*i.e.* not part of or fused to other polypeptides), or one or more fragments may be present in a single polypeptide.

The term "soluble CD164" as used herein refers to an intact or fragment of CD164 that lacks a transmembrane domain or that is not bound to a membrane.

The term "inflammation or/and immune-related activity" as used herein refers to at least one, and preferably all, of the activities described herein for soluble CD164

polypeptides. Assays for the determination of these activities are provided herein (*e.g.* Example 3), and equivalent assays can be designed by those with ordinary skill in the art.

The invention is drawn, *inter alia*, to isolated, purified or recombinant CD164 soluble polypeptides, whether fragments or full length. Soluble CD164 polypeptides of the invention are useful for reducing inflammatory responses and modulating immune response for treatment or prevention of inflammation or/and immune-related diseases and disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis.

Soluble CD164 polypeptides are also useful *inter alia* in screening assays for agonists or antagonists of CD164 activity; for raising CD164 polypeptide-specific antibodies; and in diagnostic assays. When used for the treatment or prevention of inflammation or/and immune-related diseases, disorders, or conditions, one or more soluble CD164 polypeptide(s) can be provided to a subject. Thus, various soluble fragments or soluble full-length variants of CD164 can be combined into a "cocktail" for use in the various treatment regimens.

Soluble CD164 polypeptides of the invention include variants, fragments, analogs and derivatives of the CD164 polypeptide, including modified soluble CD164 polypeptide fragments.

In other embodiments, the soluble CD164 is an isoform, mutein, fused protein, recombinant protein, functional derivative, fragment, hybrid, variant, active fraction or salt thereof and referred to as "substance(s) of the invention".

Variants-muteins

It will be recognized by one of ordinary skill in the art that some amino acids of the soluble CD164 sequence of the present invention can be varied without significant effect on the structure or function of the protein; there will be critical amino acids in the fragment sequence that determine activity. Thus, the invention further includes variants of the soluble CD164 polypeptide that have inflammation or immune-related activity as described above. Such variants include soluble CD164 sequences with one or more amino acid deletions, insertions, inversions, repeats,

and substitutions either from natural mutations or human manipulation selected according to general rules known in the art so as to have little effect on activity. Guidance concerning how to make phenotypically silent amino acid substitutions is provided below.

There are two main approaches for studying the tolerance of an amino acid sequence to change (*see*, Bowie, et al. (1990) Science, 247, 1306-10). The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

These studies have revealed that proteins are surprisingly tolerant of amino acid substitutions and indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described by Bowie et al. (supra) and the references cited therein.

Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Phe; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between the amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe, Tyr. In addition, the following groups of amino acids generally represent equivalent changes: (1) Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr; (2) Cys, Ser, Tyr, Thr; (3) Val, Ile, Leu, Met, Ala, Phe; (4) Lys, Arg, His; (5) Phe, Tyr, Trp, His.

Similarly, amino acids in the soluble CD164 polypeptide fragment sequence of the invention that are essential for function can also be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (*see, e.g.*, Cunningham, et al. (1989) Science 244(4908):1081-5). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for inflammation or/and immune-related activity using assays. Of special interest are substitutions of charged amino acids with other charged or neutral amino acids that may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical or physiologically acceptable formulations, because aggregates can be immunogenic (*see, e.g.*, Pinckard, et al., (1967) Clin. Exp. Immunol 2:331-340; Robbins, et al.,

(1987) Diabetes Jul;36(7):838-41; and Cleland, et al., (1993) Crit Rev Ther Drug Carrier Syst. 10(4):307-77).

Thus, the fragment, derivative, analog, or homolog of the soluble CD164 of the present invention may be, for example: (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code (i.e. may be a non-naturally occurring amino acid); or (ii) one in which one or more of the amino acid residues includes a substituent group; or (iii) one in which the soluble CD164 is fused with another compound, such as a compound to increase the half-life of the fragment (for example, polyethylene glycol); or (iv) one in which the additional amino acids are fused to the above form of the fragment , such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the fragment or a pro-protein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a soluble CD164 polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, not more than 40 conservative amino acid substitutions, not more than 30 conservative amino acid substitutions, and not more than 20 conservative amino acid substitutions. Also provided are polypeptides which comprise the amino acid sequence of a soluble CD164, having at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

Another specific embodiment of a modified soluble CD164 of the invention is a polypeptide that is resistant to proteolysis, for example a soluble CD164 in which a -CONH- peptide bond is modified and replaced by one or more of the following: a (CH2NH) reduced bond; a (NHCO) retro inverso bond; a (CH2-O) methylene-oxy bond; a (CH2-S) thiomethylene bond; a (CH2CH2) carba bond; a (CO-CH2) cetomethylene bond; a (CHOH-CH2) hydroxyethylene bond); a (N-N) bound; a E-alcene bond; or a -CH=CH- bond. Thus, the invention also encompasses a soluble CD164 or a variant thereof in which at least one peptide bond has been modified as described above.

In addition, amino acids have chirality within the body of either L or D. In some embodiments it is preferable to alter the chirality of the amino acids in the soluble CD164 polypeptide of the invention in order to extend half-life within the

body. Thus, in some embodiments, one or more of the amino acids are preferably in the L configuration. In other embodiments, one or more of the amino acids are preferably in the D configuration.

As used herein the term "muteins" refers to analogs of a substance according to the invention, in which one or more of the amino acid residues of a natural substance of the invention are replaced by different amino acid residues, or are deleted, or one or more amino acid residues are added to the natural sequence of substance of the invention, without changing considerably the activity of the resulting products as compared to the wild type substance of the invention. These muteins are prepared by known synthesis and/or by site-directed mutagenesis techniques, or any other known technique suitable therefor.

Any such mutein preferably has a sequence of amino acids sufficiently duplicative of that of a substance of the invention, such as to have substantially similar or even better activity to a substance of the invention.

Muteins of a substance of the invention, which can be used in accordance with the present invention, or nucleic acid coding thereof, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein.

An isoform, mutein, fused protein, recombinant protein, functional derivative, hybrid, variant, active fraction or salt thereof of a substance of the invention can be routinely obtained by one of ordinary skill in the art, without undue experimentation.

Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of polypeptides or proteins of the invention, may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule. It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, *e.g.*, under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e.g., cysteine residues. Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II;

and most preferably the synonymous amino acid groups are those defined in Table III.

TABLE I

Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

TABLE II

More Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	His, Lys, Arg
Leu	Leu, Ile, Phe, Met
Pro	Ala, Pro
Thr	Thr
Ala	Pro, Ala
Val	Val, Met, Ile
Gly	Gly
Ile	Ile, Met, Phe, Val, Leu
Phe	Met, Tyr, Ile, Leu, Phe
Tyr	Phe, Tyr
Cys	Cys, Ser
His	His, Gln, Arg
Gln	Glu, Gln, His
Asn	Asp, Asn
Lys	Lys, Arg
Asp	Asp, Asn
Glu	Glu, Gln
Met	Met, Phe, Ile, Val, Leu
Trp	Trp

TABLE III

Most Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	Arg
Leu	Leu, Ile, Met

Pro	Pro
Thr	Thr
Ala	Ala
Val	Val
Gly	Gly
Ile	Ile, Met, Leu
Phe	Phe
Tyr	Tyr
Cys	Cys, Ser
His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
Glu	Glu
Met	Met, Ile, Leu
Trp	Met

Examples of production of amino acid substitutions in proteins which can be used for obtaining muteins a substance of the invention, for use in the present invention include any known method steps, such as presented in US patents 4,959,314, 4,588,585 and 4,737,462, to Mark et al; 5,116,943 to Koths et al., 4,965,195 to Namen et al; 4,879,111 to Chong et al; and 5,017,691 to Lee et al; and lysine substituted proteins presented in US patent No. 4,904,584 (Shaw et al). Specific muteins of IFN-β have been described, for example by Mark et al., 1984.

Percent Identity

The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 50% identical, at least 60% identical, or 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a soluble CD164 as described above. By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a soluble CD164 amino acid sequence is meant that the amino acid sequence is identical to the soluble CD164 sequence except that

it may include up to five amino acid alterations per each 100 amino acids of the soluble CD164 polypeptide amino acid sequence. Thus, to obtain a polypeptide having an amino acid sequence at least 95% identical to a soluble CD164 amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the sequence may be inserted, deleted, or substituted with another amino acid compared with the soluble CD164 polypeptide sequence. These alterations may occur at the amino or carboxy termini or anywhere between those terminal positions, interspersed either individually among residues in the sequence or in one or more contiguous groups within the sequence.

As a practical matter, whether any particular polypeptide is a percentage identical to a soluble CD164 can be determined conventionally using known computer programs. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, (1988) Proc Natl Acad Sci USA 85(8):2444-8; Altschul et al., (1990) J Mol Biol 215(3):403-410; Thompson et al., (1994) Nucleic Acids Res 22(2):4673-4680; Higgins et al., (1996) Meth Enzymol 266:383-402; Altschul et al., (1997) Nuc Acids Res 25:3389-3402; Altschul et al., (1993) Nature Genetics 3:266-272). In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art (*See, e.g.*, Karlin and Altschul (1990) Proc Natl Acad Sci USA 87(6):2264-8; Altschul et al., 1990, 1993, 1997, all supra). In particular, five specific BLAST programs are used to perform the following tasks:

(1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;

(2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

(3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

(4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

(5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (*i.e.*, aligned) by means of a scoring matrix, many of

which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (see, Gonnet et al., (1992) Science 256(5062):1443-5; Henikoff and Henikoff (1993) Proteins 17(1):49-61). Less preferably, the PAM or PAM250 matrices may also be used (*See, e.g.*, Schwartz and Dayhoff, eds, (1978) Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure, Washington: National Biomedical Research Foundation). The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent homology. Preferably, the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (*See, e.g.*, Karlin and Altschul, (1990) Proc Natl Acad Sci USA 87(6):2264-8). The BLAST programs may be used with the default parameters or with modified parameters provided by the user. Preferably, the parameters are default parameters.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (1990) Comp. App. Biosci. 6:237-245. In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group=25 Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=247 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N-or C-terminal deletions, not because of internal deletions, the results, in percent identity, must be manually corrected because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C- terminal of the subject sequence, that are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues

to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query amino acid residues outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100-residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not match/align with the first residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%.

In another example, a 90-residue subject sequence is compared with a 100-residue query sequence. This time the deletions are internal so there are no residues at the N- or C-termini of the subject sequence, which are not matched/aligned with the query. In this case, the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected. No other manual corrections are made for the purposes of the present invention.

Production

Soluble CD164 polypeptides are preferably isolated from human or mammalian tissue samples or expressed from human or mammalian genes in human or mammalian cells. The soluble CD164 polypeptides of the invention can be made using routine expression methods known in the art. The polynucleotide encoding the desired polypeptide fragments is ligated into an expression vector suitable for any convenient host. Both eukaryotic and prokaryotic host systems are used in forming recombinant polypeptide fragments. The polypeptide fragment is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification is by any technique known in the art, for example, differential extraction, salt fractionation, chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins.

In an alternative embodiment, the polypeptides of the invention are isolated from milk. Any of a large number of methods can be used to purify the present polypeptides from milk, including those taught in Protein Purification Applications, A Practical Approach (New Edition), Edited by Simon Roe, AEA Technology Products

and Systems, Biosciences, Harwell; Clark (1998) J Mammary Gland Biol Neoplasia 3:337-50; Wilkins and Velander (1992) 49:333-8; U.S. Patent Nos. 6,140,552; 6,025,540; Hennighausen, Protein Expression and Purification, vol. 1, pp. 3-8 (1990); Harris et al. (1997) Bioseparation 7:31-7; Degener et al. (1998) J Chromatog 799:125-37; Wilkins (1993) J Cell Biochem Suppl. 0 (17 part A):39; the entire disclosures of each of which are herein incorporated by reference. In a typical embodiment, milk is centrifuged, e.g. at a relatively low speed, to separate the lipid fraction, and the aqueous supernatant is then centrifuged at a higher speed to separate the casein in the milk from the remaining, "whey" fraction. Often, biomedical proteins are found in this whey fraction, and can be isolated from this fraction using standard chromatographic or other procedures commonly used for protein purification, e.g. as described elsewhere in the present application. In one preferred embodiment, CD164 polypeptides are purified using antibodies specific to CD164 polypeptides, e.g. using affinity chromatography. In addition, methods can be used to isolate particular CD164 fragments, e.g. electrophoretic or other methods for isolating proteins of a particular size. The CD164 polypeptides isolating using these methods can be naturally occurring or can be the result of the recombinant production of the protein in the mammary glands of a non-human mammal, as described infra. In one such embodiment, the CD164 is produced as a fusion protein with a heterologous, antigenic polypeptide sequence, which antigenic sequence can be used to purify the protein, e.g., using standard immuno-affinity methodology.

In addition, shorter protein fragments may be produced by chemical synthesis. Alternatively, the proteins of the invention are extracted from cells or tissues of humans or non-human animals. Methods for purifying proteins are known in the art, and include the use of detergents or chaotropic agents to disrupt particles followed by differential extraction and separation of the polypeptides by ion exchange chromatography, affinity chromatography, sedimentation according to density, and gel electrophoresis.

Any CD164 cDNA can be used to express CD164 polypeptides. The nucleic acid encoding CD164 to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology.

The expression vector is any of the mammalian, yeast, insect or bacterial expression systems known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Genetics Institute (Cambridge, MA), Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence can be

optimized for the particular expression organism into which the expression vector is introduced, as explained by Hatfield, et al., US Patent Number 5,082,767, the disclosures of which are incorporated by reference herein in their entirety.

If the nucleic acid encoding a soluble CD164 polypeptide lacks a methionine to serve as the initiation site, an initiating methionine can be introduced next to the first codon of the nucleic acid using conventional techniques. sSimilarly, if the insert from the soluble CD164 polypeptide cDNA lacks a poly A signal, this sequence can be added to the construct by, for example, splicing out the Poly A signal from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex Thymidine Kinase promoter and the selectable neomycin gene.

The nucleic acid encoding a soluble CD164 can be obtained by PCR from a vector containing the soluble CD164 nucleotide sequence using oligonucleotide primers complementary to the desired CD164 cDNA and containing restriction endonuclease sequences for Pst I incorporated into the 5' primer and BglII at the 5' end of the corresponding cDNA 3' primer, taking care to ensure that the sequence encoding the soluble CD164 is positioned properly with respect to the poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and digested with BglII.

Transfection of a soluble CD164 expressing vector into mouse NIH 3T3 cells is one embodiment of introducing polynucleotides into host cells. Introduction of a polynucleotide encoding a polypeptide into a host cell can be effectuated by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al. ((1986) Methods in Molecular Biology, Elsevier Science Publishing Co., Inc., Amsterdam). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention (i.e. a soluble CD164) can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC")

is employed for purification. Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells.

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Preferably soluble CD164 polypeptides of the invention are glycosylated. Still preferably, the soluble CD164 polypeptides of the invention are glycosylated at positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (*e.g.*, coding sequence), and/or to include genetic material (*e.g.*, heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (*e.g.*, promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, see, *e.g.*, US Patent Number 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., (1989) Proc Natl Acad Sci USA 86(22):8932-5; Koller et al., (1989) Proc Natl Acad Sci USA 86(22):8927-31; and Zijlstra et al. (1989) Nature 342(6248):435-8; the disclosures of each of which are incorporated by reference in their entireties).

Modifications

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (*See, e.g.*, Creighton, 1983 Proteins. New York, New

York: W.H. Freeman and Company; and Hunkapiller et al., (1984) Nature 310(5973):105-11). For example, a relative short fragment of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the fragment sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoroamino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptide fragments which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, myristoylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptide fragments may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the polypeptide.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention that may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity. See U.S. Patent No: 4,179,337. The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions

within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the polypeptide with consideration of effects on functional or antigenic domains of the polypeptide. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al. (1992) Exp Hematol 20(8):1028-35, reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary

amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

II. CD164 Polynucleotides of the Invention

Preferred polynucleotides are those that encode CD164 polypeptides of the invention. The recombinant polynucleotides encoding CD164 polypeptides can be used in a variety of ways, including, but not limited to, expressing the polypeptide in recombinant cells for use in screening assays for antagonists and agonists of its activity as well as to facilitate its purification for use in a variety of ways including, but not limited to screening assays for agonists and antagonists of its activity, diagnostic screens, and raising antibodies, as well as treatment and/or prevention of inflammation or autoimmune diseases and disorders.

The invention relates to the polynucleotides encoding CD164 polypeptides and variant polypeptides thereof as described herein. These polynucleotides may be purified, isolated, and/or recombinant. In all cases, the desired CD164 polynucleotides of the invention are those that encode CD164 polypeptides of the invention having inflammation- or autoimmune-related activity as described and discussed herein.

Fragments

A polynucleotide fragment is a polynucleotide having a sequence that entirely is the same as part, but not all, of a CD164 polypeptide. Such fragments may be "free-standing", *i.e.* not part of or fused to other polynucleotides, or they may be comprised within another CD164 (heterologous) polynucleotide of which they form a part or region. However, several CD164 polynucleotide fragments may be comprised within a single polynucleotide.

Variants

In other preferred embodiments, variants of CD164 polynucleotides encoding CD164 polypeptides are envisioned. Variants of polynucleotides, as the term is used herein, are polynucleotides whose sequence differs from a reference polynucleotide. A variant of a polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

Polynucleotide variants that comprise a sequence substantially different from those described above but that, due to the degeneracy of the genetic code, still encode CD164 polypeptides of the present invention are also specifically envisioned. It would also be routine for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (*e.g.,* change codons in the human mRNA to those preferred by other mammalian or bacterial host cells).

As stated above, variant polynucleotides may occur naturally, such as a natural allelic variant, or by recombinant methods. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (See, *e.g.,* B. Lewin, (1990) Genes IV, Oxford University Press, New York). Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Such nucleic acid variants include those produced by nucleotide substitutions, deletions, or additions. The substitutions, deletions, or additions may involve one or more nucleotides. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of a CD164 polypeptide of the invention. Also preferred in this regard are conservative substitutions.

Nucleotide changes present in a variant polynucleotide are preferably silent, which means that they do not alter the amino acids encoded by the polynucleotide. However, nucleotide changes may also result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence.

In cases where the nucleotide substitutions result in one or more amino acid changes, preferred soluble CD164 polypeptides include those that retain one or more inflammation- or/and autoimmune-related activity.

By "retain the same activities" is meant that the activity measured using the polypeptide encoded by the variant CD164 polynucleotide in assays is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, and not more than 101%, 102%, 103%, 104%, 105%, 110%, 115%, 120% or 125% of the activity measured using a soluble CD164 as defined by any of SEQ ID NO: 1 or SEQ ID NO: 6.

By the activity being "increased" is meant that the activity measured using the polypeptide encoded by the variant CD164 polynucleotide in assays is at least 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 170%, 180%, 190%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 450%, or 500% of the activity

measured using a soluble CD164 as defined by any of SEQ ID NO: 1 or SEQ ID NO: 6.

By the activity being "decreased" is meant that the activity measured using the polypeptide encoded by the variant CD164 polynucleotide in assays is decreased by at least 25%, 30%, 35%, 40%, 45%, or 50% of the activity measured using a soluble CD164 as defined by any of SEQ ID NO: 1 or SEQ ID NO: 6.

Percent Identity

The present invention is further directed to nucleic acid molecules having sequences at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequences of SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 3 or SEQ ID NO: 4 or SEQ ID NO: 5 or SEQ ID NO: 6 or fragments thereof that encode a polypeptide having inflammation- or/and autoimmune-related activity as described in Section I of the Preferred Embodiments of the Invention. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequences shown in SEQ ID NO: 1 or SEQ ID NO: 2 or SEQ ID NO: 3 or SEQ ID NO: 4 or SEQ ID NO: 5 or SEQ ID NO: 6 or fragments thereof will encode a polypeptide having biological activity. In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having biological activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly affect protein function (*e.g.*, replacing one aliphatic amino acid with a second aliphatic amino acid).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to a CD164 sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the CD164 nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted, inserted, or substituted with another nucleotide. The query sequence may be an entire sequence or any fragment specified as described herein.

The methods of determining and defining whether any particular nucleic acid molecule or polypeptide is at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%

or 99% identical to a nucleotide sequence of the present invention can be done by using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., ((1990) Comput Appl Biosci 6(3):237-45). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by first converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only nucleotides outside the 5' and 3' nucleotides of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90-nucleotide subject sequence is aligned to a 100-nucleotide query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 nucleotides at 5' end. The 10 unpaired nucleotides represent 10% of the sequence (number of nucleotides at the 5' and 3' ends not matched/total number of nucleotides in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 nucleotides were perfectly matched the final percent identity would be 90%.

In another example, a 90 nucleotide subject sequence is compared with a 100 nucleotide query sequence. This time the deletions are internal deletions so that there are no nucleotides on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only nucleotides 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of the present invention.

Fusions

Further included in the present invention are polynucleotides encoding the polypeptides of the present invention that are fused in frame to the coding sequences for additional heterologous amino acid sequences. Also included in the present invention are nucleic acids encoding polypeptides of the present invention together with additional, non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, vector sequence, sequences used for purification, probing, or priming. For example, heterologous sequences include transcribed, non-translated sequences that may play a role in transcription, and mRNA processing, for example, ribosome binding and stability of mRNA. The heterologous sequences may alternatively comprise additional coding sequences that provide additional functionalities. Thus, a nucleotide sequence encoding a polypeptide may be fused to a tag sequence, such as a sequence encoding a peptide that facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the tag amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. For instance, hexa-histidine provides for convenient purification of the fusion protein (See, Gentz et al., (1989) Proc Natl Acad Sci USA 86(3):821-4). The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein (See, Wilson et al., (1984) Cell 37(3):767-78). As discussed above, other such fusion proteins include CD164 cDNA fused to Fc at the N- or C-terminus.

In other words, the term "fused protein" refers to a polypeptide comprising a substance of the invention, or a mutein thereof, fused to another protein, which *e.g.*, has an extended residence time in body fluids. A substance of the invention may thus be fused to another protein, polypeptide or the like, *e.g.*, an immunoglobulin or a fragment thereof.

"Functional derivatives" as used herein cover derivatives of a substance of the invention, and their muteins and fused proteins, which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, *i.e.* they do not destroy the activity of the protein which is substantially similar to the activity a substance of the invention, and do not confer toxic properties on compositions containing it. These derivatives may, for example, include polyethylene glycol side-chains, which may mask antigenic sites and extend the residence of a substance of the invention in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (*e.g.* alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "active fractions" of a substance of the invention, or muteins and fused proteins, the present invention covers any fragment or precursors of the polypeptide chain of the protein molecule alone or together with associated molecules or residues linked thereto, *e.g.*, sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said fraction has no significantly reduced activity as compared to the corresponding substance of the invention.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the proteins described above or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Of course, any such salts must retain the biological activity of the proteins relevant to the present invention, i.e., the ability to bind to the corresponding receptor and initiate receptor signaling.

In a further preferred embodiment, the fused protein comprises an Ig fusion. The fusion may be direct, or via a short linker peptide which can be as short as 1 to 3 amino acid residues in length or longer, for example, 13 amino acid residues in length. Said linker may be a tripeptide of the sequence E-F-M (Glu-Phe-Met), for

example, or a 13-amino acid linker sequence comprising Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met introduced between the sequence of the substances of the invention and the immunoglobulin sequence. The resulting fusion protein has improved properties, such as an extended residence time in body fluids (half-life), increased specific activity, increased expression level, or the purification of the fusion protein is facilitated.

In a preferred embodiment, CD164 is fused to the constant region of an Ig molecule. Preferably, it is fused to heavy chain regions, like the CH2 and CH3 domains of human IgG1, for example. Other isoforms of Ig molecules are also suitable for the generation of fusion proteins according to the present invention, such as isoforms IgG_2 or IgG_4, or other Ig classes, like IgM or IgA, for example. Fusion proteins may be monomeric or multimeric, hetero- or homomultimeric.

In a further preferred embodiment, the functional derivative comprises at least one moiety attached to one or more functional groups, which occur as one or more side chains on the amino acid residues. Preferably, the moiety is a polyethylene (PEG) moiety. PEGylation may be carried out by known methods, such as the ones described in WO99/55377, for example.

III. Recombinant Vectors of the Invention

The term "vector" is used herein to designate either a circular or a linear DNA or RNA molecule, that is either double-stranded or single-stranded, and that comprises at least one polynucleotide of interest that is sought to be transferred in a cell host or in a unicellular or multicellular host organism.

The present invention relates to recombinant vectors comprising any one of the polynucleotides described herein.

The present invention encompasses a family of recombinant vectors that comprise polynucleotides encoding CD164 polypeptides of the invention.

In a first preferred embodiment, a recombinant vector of the invention is used to amplify the inserted polynucleotide in a suitable cell host, this polynucleotide being amplified every time that the recombinant vector replicates. The inserted polynucleotide can be one that encodes CD164 polypeptides of the invention.

A second preferred embodiment of the recombinant vectors according to the invention consists of expression vectors comprising polynucleotides encoding CD164 polypeptides of the invention. Within certain embodiments, expression vectors are employed to express a soluble CD164 fragment of the invention, preferably a modified soluble CD164 fragment, which can be then purified and, for example, be used as a treatment for inflammation- or/and autoimmune related diseases.

Expression requires that appropriate signals are provided in the vectors, said signals including various regulatory elements, such as enhancers/promoters from both viral and mammalian sources, that drive expression of the genes of interest in host cells. Dominant drug selection markers for establishing permanent, stable, cell clones expressing the products are generally included in the expression vectors of the invention, as they are elements that link expression of the drug selection markers to expression of the polypeptide.

More particularly, the present invention relates to expression vectors which include nucleic acids encoding a CD164 polypeptide, or a modified CD164 as described herein, or variants or fragments thereof, under the control of a regulatory sequence selected among CD164 polypeptides, or alternatively under the control of an exogenous regulatory sequence.

Consequently, preferred expression vectors of the invention are selected from the group consisting of: (a) a CD164 regulatory sequence and driving the expression of a coding polynucleotide operably linked thereto; and (b) an CD164 coding sequence, operably linked to regulatory sequences allowing its expression in a suitable cell host and/or host organism.

Some of the elements that can be found in the vectors of the present invention are described in further detail in the following sections.

1) General features of the expression vectors of the invention:

A recombinant vector according to the invention comprises, but is not limited to, a YAC (Yeast Artificial Chromosome), a BAC (Bacterial Artificial Chromosome), a phage, a phagemid, a cosmid, a plasmid, or even a linear DNA molecule which may consist of a chromosomal, non-chromosomal, semi-synthetic or synthetic DNA. Such a recombinant vector can comprise a transcriptional unit comprising an assembly of :

(1) a genetic element or elements having a regulatory role in gene expression, for example promoters or enhancers. Enhancers are cis-acting elements of DNA, usually from about 10 to 300 bp in length that act on the promoter to increase the transcription;

(2) a structural or coding sequence which is transcribed into mRNA and eventually translated into a polypeptide, said structural or coding sequence being operably linked to the regulatory elements described in (1); and

(3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, when a recombinant protein is expressed without a leader or transport sequence, it may include a N-terminal residue. This residue may or may not be

subsequently cleaved from the expressed recombinant protein to provide a final product.

Generally, recombinant expression vectors will include origins of replication, selectable markers permitting transformation of the host cell, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably a leader sequence capable of directing secretion of the translated protein into the periplasmic space or the extracellular medium. In a specific embodiment wherein the vector is adapted for transfecting and expressing desired sequences in mammalian host cells, preferred vectors will comprise an origin of replication in the desired host, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5'-flanking non-transcribed sequences. DNA sequences derived from the SV40 viral genome, for example SV40 origin, early promoter, enhancer, splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

2) Regulatory elements

Promoters

The suitable promoter regions used in the expression vectors of the present invention are chosen taking into account the cell host in which the heterologous gene is expressed. The particular promoter employed to control the expression of a nucleic acid sequence of interest is not believed to be important, so long as it is capable of directing the expression of the nucleic acid in the targeted cell. Thus, where a human cell is targeted, it is preferable to position the nucleic acid coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell, such as, for example, a human or a viral promoter. The promoter used may be constitutive or inducible.

A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed. Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

Promoter regions can be selected from any desired gene using, for example, CAT (chloramphenicol transferase) vectors and more preferably pKK232-8 and pCM7 vectors.

Preferred bacterial promoters are the LacI, LacZ, the T3 or T7 bacteriophage RNA polymerase promoters, the gpt, lambda PR, PL and trp promoters (EP 0036776), the polyhedrin promoter, or the p10 protein promoter from baculovirus (Kit Novagen) (Smith et al., (1983) Mol Cell Biol 3(12):2156-65; O'Reilly et al., 1992), the lambda PR promoter or also the trc promoter.

Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-L. In addition, promoters specific for a particular cell type may be chosen, such as those facilitating expression in adipose tissue, muscle tissue, or liver. Selection of a convenient vector and promoter is well within the level of ordinary skill in the art.

The choice of a promoter is well within the ability of a person skilled in the field of genetic engineering. For example, one may refer to Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989), or also to the procedures described by Fuller et al. (1996) Immunology in Current Protocols in Molecular Biology.

Other regulatory elements

Where a cDNA insert is employed, one will typically desire to include a polyadenylation signal to effect proper polyadenylation of the gene transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and any such sequence may be employed such as human growth hormone and SV40 polyadenylation signals. Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

Vectors containing the appropriate DNA sequence as described above can be utilized to transform an appropriate host to allow the expression of the desired polypeptide or polynucleotide.

3) Selectable markers

Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression construct. The selectable marker genes for selection of transformed host cells are preferably dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, TRP1 for S. cerevisiae or tetracycline, rifampicin or ampicillin resistance in *E. coli*, or levan saccharase for mycobacteria, this latter marker being a negative selection marker.

4) Preferred vectors

Bacterial vectors

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and a bacterial origin of replication derived from commercially available plasmids comprising genetic elements of pBR322 (ATCC 37017). Such commercial vectors include, but are not limited to, pKK223-3 (Pharmacia, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA).

Large numbers of other suitable vectors are known to those of skill in the art, and are commercially available, such as the following bacterial vectors : pTrc-His, pET30-His, pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); pQE-30 (QIAexpress).

Baculovirus vectors

A suitable vector for the expression of polypeptides of the invention is a baculovirus vector that can be propagated in insect cells and in insect cell lines. A specific suitable host vector system is the pVL1392/1393 baculovirus transfer vector (Pharmingen) that is used to transfect the SF9 cell line (ATCC N°CRL 1711) which is derived from Spodoptera frugiperda.

Further suitable baculovirus vectors are known to those skilled in the art, for example, FastBacHT. Other suitable vectors for the expression of an APM1 globular head polypeptide in a baculovirus expression system include, but are not limited to, those described by Chai et al. (1993; Biotechnol Appl Biochem. Dec;18 (Pt 3):259-73); Vlasak et al. (1983; Eur J Biochem Sep 1;135(1):123-6); and Lenhard et al. (1996; Gene Mar 9;169(2):187-90).

Mammalian vectors

Further suitable vectors for the expression of polypeptides of the invention are mammalian vectors. A number of suitable vector systems are known to those skilled in the art, for example, pcDNA4HisMax, pcDNA3.1Hygro-His and pcDNA3.1Hygro.

Viral vectors

In one specific embodiment, the vector is derived from an adenovirus. Preferred adenovirus vectors according to the invention are those described by Feldman and Steg (1996; Semin Interv Cardiol 1(3):203-8) or Ohno et al. (1994; Science 265(5173):781-4). Another preferred recombinant adenovirus according to this specific embodiment of the present invention is the human adenovirus type 2 or

5 (Ad 2 or Ad 5) or an adenovirus of animal origin (French patent application No. FR-93.05954).

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery systems of choice for the transfer of exogenous polynucleotides *in vivo*, particularly to mammals, including humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host.

Particularly preferred retroviruses for the preparation or construction of retroviral *in vitro* or *in vivo* gene delivery vehicles of the present invention include retroviruses selected from the group consisting of Mink-Cell Focus Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus and Rous Sarcoma virus. Particularly preferred Murine Leukemia Viruses include the 4070A and the 1504A viruses, Abelson (ATCC No VR-999), Friend (ATCC No VR-245), Gross (ATCC No VR-590), Rauscher (ATCC No VR-998) and Moloney Murine Leukemia Virus (ATCC No VR-190; PCT Application No WO 94/24298). Particularly preferred Rous Sarcoma Viruses include Bryan high titer (ATCC Nos VR-334, VR-657, VR-726, VR-659 and VR-728). Other preferred retroviral vectors are those described in Roth et al. (1996), PCT Application No WO 93/25234, PCT Application No WO 94/ 06920, Roux et al., ((1989) Proc Natl Acad Sci USA 86(23):9079-83), Julan et al., (1992) J. Gen. Virol. 3:3251-3255 and Neda et al. ((1991) J Biol Chem 266(22):14143-6).

Yet another viral vector system that is contemplated by the invention consists of the adeno-associated virus (AAV). The adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al., (1992) Curr Top Microbiol Immunol 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (Flotte et al., (1992) Am J Respir Cell Mol Biol 7(3):349-56; Samulski et al., (1989) J Virol 63(9):3822-8; McLaughlin et al., (1989) Am J Hum Genet 59:561-569). One advantageous feature of AAV derives from its reduced efficacy for transducing primary cells relative to transformed cells.

5) Delivery of the recombinant vectors

In order to effect expression of the polynucleotides of the invention, these constructs must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cell lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states.

One mechanism is viral infection where the expression construct is encapsulated in an infectious viral particle.

Several non-viral methods for the transfer of polynucleotides into cultured mammalian cells are also contemplated by the present invention, and include, without being limited to, calcium phosphate precipitation (Graham et al., (1973) Virology 54(2):536-9; Chen et al., (1987) Mol Cell Biol 7(8):2745-52), DEAE-dextran (Gopal, (1985) Mol Cell Biol 5(5):1188-90), electroporation (Tur-Kaspa et al., (1986) Mol Cell Biol 6(2):716-8; Potter et al., (1984) Proc Natl Acad Sci USA 81(22):7161-5.), direct microinjection (Harland et al., (1985) J Cell Biol 101(3):1094-9), DNA-loaded liposomes (Nicolau et al., (1982) Biochim Biophys Acta 721(2):185-90; Fraley et al., (1979) Proc Natl Acad Sci USA 76(7):3348-52), and receptor-mediated transfection (Wu and Wu, (1987) J Biol Chem 262(10):4429-32; Wu and Wu (1988) Biochemistry 27(3):887-92). Some of these techniques may be successfully adapted for *in vivo* or *ex vivo* use.

Once the expression polynucleotide has been delivered into the cell, it may be stably integrated into the genome of the recipient cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle.

Another possibility of carrying out the present invention is to activate endogenously the genes for the compounds of the invention. In this case, a vector for inducing and/or enhancing the endogenous production of CD164 and decreasing or inhibiting the endogeneous production of e.g. soluble CD164 in a cell normally silent for expression of soluble CD164, or which expresses amounts of soluble CD164 which are not sufficient, is used for treatment of inflammation and/or autoimmune disorders. The vector may comprise regulatory sequences functional in the cells desired to express CD164 and/or repress CD164. Such regulatory sequences in the case of CD164 may be promoters or enhancers. The regulatory sequence may then be introduced into the right locus of the genome by homologous recombination, thus operably linking the regulatory sequence with the gene, the expression of which is required to be induced or enhanced. The technology is usually referred to as "endogenous gene activation" (E.G.A), and it is described e.g. in WO 91/09955.

One specific embodiment for a method for delivering a protein or peptide to the interior of a cell of a vertebrate *in vivo* comprises the step of introducing a preparation comprising a physiologically acceptable carrier and a naked

polynucleotide operatively coding for the polypeptide of interest into the interstitial space of a tissue comprising the cell, whereby the naked polynucleotide is taken up into the interior of the cell and has a physiological effect. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* as well.

Compositions for use *in vitro* and *in vivo* comprising a "naked" polynucleotide are described in PCT application No. WO 90/11092 (Vical Inc.) and also in PCT application No. WO 95/11307 (Institut Pasteur, INSERM, Université d'Ottawa) as well as in the articles of Tascon et al. (1996) Nature Medicine 2(8):888-892 and of Huygen et al. ((1996) Nat Med 2(8):893-8).

In still another embodiment of the invention, the transfer of a naked polynucleotide of the invention, including a polynucleotide construct of the invention, into cells may be proceeded with a particle bombardment (biolistic), said particles being DNA-coated microprojectiles accelerated to a high velocity allowing them to pierce cell membranes and enter cells without killing them, such as described by Klein et al. ((1990) Curr Genet Feb;17(2):97-103).

In a further embodiment, the polynucleotide of the invention may be entrapped in a liposome (Ghosh and Bacchawat, (1991) Targeted Diagn Ther 4:87-103; Wong et al., (1980) Gene 10:87-94; Nicolau et al., (1987) Methods Enzymol 149:157-76). These liposomes may further be targeted to cells expressing LSR by incorporating leptin, triglycerides, ACRP30, or other known LSR ligands into the liposome membrane.

The amount of vector to be injected to the desired host organism varies according to the site of injection. As an indicative dose, it will be injected between 0.1 and 100 μg of the vector in an animal body, preferably a mammal body, for example a mouse body.

In another embodiment of the vector according to the invention, it may be introduced *in vitro* in a host cell, preferably in a host cell previously harvested from the animal to be treated and more preferably a somatic cell such as a muscle cell. In a subsequent step, the cell that has been transformed with the vector coding for the desired CD164 polypeptide or the desired fragment thereof is reintroduced into the animal body in order to deliver the recombinant protein within the body either locally or systemically.

IV. Recombinant Cells of the Invention

Another object of the invention consists of host cells recombinant for, i.e., that have been transformed or transfected with one of the polynucleotides described herein, and more precisely a polynucleotide comprising a polynucleotide encoding a CD164 polypeptide of the invention such as any one of those described in

"Polynucleotides of the Invention". These polynucleotides can be present in cells as a result of transient or stable transfection. The invention includes host cells that are transformed (prokaryotic cells) or that are transfected (eukaryotic cells) with a recombinant vector such as any one of those described in "Recombinant Vectors of the Invention".

The invention further relates to the use of a cell that has been genetically modified to produce a soluble CD164 in the manufacture of a medicament for the treatment and/or prevention of inflammation and/or autoimmune disorders.

Generally, a recombinant host cell of the invention comprises at least one of the polynucleotides or the recombinant vectors of the invention that are described herein.

Preferred host cells used as recipients for the recombinant vectors of the invention are the following :

a) Prokaryotic host cells: *Escherichia coli* strains (I.E: DH5-α strain), *Bacillus subtilis*, *Salmonella typhimurium*, and strains from species like *Pseudomonas*, *Streptomyces* and *Staphylococcus*, and

b) Eukaryotic host cells: HeLa cells (ATCC N°CCL2; N°CCL2.1; N°CCL2.2), Cv 1 cells (ATCC N°CCL70), COS cells (ATCC N°CRL1650; N°CRL1651), Sf-9 cells (ATCC N°CRL1711), C127 cells (ATCC N° CRL-1804), 3T3 (ATCC N° CRL-6361), CHO (ATCC N° CCL-61), human kidney 293 (ATCC N° 45504; N° CRL-1573), BHK (ECACC N° 84100501; N° 84111301), PLC cells, HepG2, and Hep3B.

The constructs in the host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

Following transformation of a suitable host and growth of the host to an appropriate cell density, the selected promoter is induced by appropriate means, such as temperature shift or chemical induction, and cells are cultivated for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known by the skilled artisan.

Further, according to the invention, these recombinant cells can be created *in vitro* or *in vivo* in an animal, preferably a mammal, most preferably selected from the group consisting of mice, rats, dogs, pigs, sheep, cattle, and primates, not to include

humans. Recombinant cells created *in vitro* can also be later surgically implanted in an animal, for example. Methods to create recombinant cells *in vivo* in animals are well known in the art.

The present invention also encompasses primary, secondary, and immortalized homologously recombinant host cells of vertebrate origin, preferably mammalian origin and particularly human origin, that have been engineered to: a) insert exogenous (heterologous) polynucleotides into the endogenous chromosomal DNA of a targeted gene, b) delete endogenous chromosomal DNA, and/or c) replace endogenous chromosomal DNA with exogenous polynucleotides. Insertions, deletions, and/or replacements of polynucleotide sequences may be to the coding sequences of the targeted gene and/or to regulatory regions, such as promoter and enhancer sequences, operably associated with the targeted gene.

The present invention further relates to a method of making a homologously recombinant host cell *in vitro* or *in vivo*, wherein the expression of a targeted gene not normally expressed in the cell is altered. Preferably the alteration causes expression of the targeted gene under normal growth conditions or under conditions suitable for producing the polypeptide encoded by the targeted gene. The method comprises the steps of: (a) transfecting the cell *in vitro* or *in vivo* with a polynucleotide construct, the polynucleotide construct comprising; (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; and (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination.

The present invention further relates to a method of altering the expression of a targeted gene in a cell *in vitro* or *in vivo* wherein the gene is not normally expressed in the cell, comprising the steps of: (a) transfecting the cell *in vitro* or *in vivo* with a polynucleotide construct, the polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; and (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and (c) maintaining the homologously recombinant cell *in vitro* or *in vivo* under conditions appropriate for expression of the gene.

The present invention further relates to a method of making a polypeptide of the present invention by altering the expression of a targeted endogenous gene in a cell *in vitro* or *in vivo* wherein the gene is not normally expressed in the cell, comprising the steps of: a) transfecting the cell *in vitro* with a polynucleotide

construct, the polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and c) maintaining the homologously recombinant cell *in vitro* or *in vivo* under conditions appropriate for expression of the gene thereby making the polypeptide.

The present invention further relates to a polynucleotide construct that alters the expression of a targeted gene in a cell type in which the gene is not normally expressed. This occurs when a polynucleotide construct is inserted into the chromosomal DNA of the target cell, wherein the polynucleotide construct comprises: a) a targeting sequence; b) a regulatory sequence and/or coding sequence; and c) an unpaired splice-donor site, if necessary. Further included are polynucleotide constructs, as described above, wherein the construct further comprises a polynucleotide that encodes a polypeptide and is in-frame with the targeted endogenous gene after homologous recombination with chromosomal DNA.

The compositions may be produced, and methods performed, by techniques known in the art, such as those described in U.S. Patent Nos: 6,054,288; 6,048,729; 6,048,724; 6,048,524; 5,994,127; 5,968,502; 5,965,125; 5,869,239; 5,817,789; 5,783,385; 5,733,761; 5,641,670; 5,580,734; International Publication Nos:WO96/29411, WO 94/12650; and scientific articles described by Koller et al., (1994) Annu. Rev. Immunol. 10:705-730; the disclosures of each of which are incorporated by reference in their entireties).

The CD164 gene expression in mammalian, and typically human, cells may be rendered defective, or alternatively it may be enhanced, with the insertion of a CD164 genomic or cDNA sequence with the replacement of the CD164 gene counterpart in the genome of an animal cell by a CD164 polynucleotide according to the invention. These genetic alterations may be generated by homologous recombination events using specific DNA constructs that have been previously described.

One kind of host cell that may be used are mammalian zygotes, such as murine zygotes. For example, murine zygotes may undergo microinjection with a purified DNA molecule of interest, for example a purified DNA molecule that has previously been adjusted to a concentration range from 1 ng/ml –for BAC inserts- 3 ng/μl –for P1 bacteriophage inserts- in 10 mM Tris-HCl, pH 7.4, 250 μM EDTA containing 100 mM NaCl, 30 μM spermine, and 70 μM spermidine. When the DNA to be microinjected has a large size, polyamines and high salt concentrations can be

used in order to avoid mechanical breakage of this DNA, as described by Schedl et al ((1993) Nature 362(6417):258-61).

Any one of the polynucleotides of the invention, including the DNA constructs described herein, may be introduced in an embryonic stem (ES) cell line, preferably a mouse ES cell line. ES cell lines are derived from pluripotent, uncommitted cells of the inner cell mass of pre-implantation blastocysts. Preferred ES cell lines are the following: ES-E14TG2a (ATCC No.CRL-1821), ES-D3 (ATCC No.CRL1934 and No. CRL-11632), YS001 (ATCC No. CRL-11776), 36.5 (ATCC No. CRL-11116). To maintain ES cells in an uncommitted state, they are cultured in the presence of growth inhibited feeder cells that provide the appropriate signals to preserve this embryonic phenotype and serve as a matrix for ES cell adherence. Preferred feeder cells are primary embryonic fibroblasts that are established from tissue of day 13- day 14 embryos of virtually any mouse strain, that are maintained in culture, such as described by Abbondanzo et al. (1993; Methods Enzymol 225:803-23) and are inhibited in growth by irradiation, such as described by Robertson ((1987) Embryo-derived stem cell lines. In: E.J. Robertson Ed. Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, IRL Press, Oxford), or by the presence of an inhibitory concentration of LIF, such as described by Pease and Williams (1990; Exp Cell Res 190(2):209-11).

The constructs in the host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

Following transformation of a suitable host and growth of the host to an appropriate cell density, the selected promoter is induced by appropriate means, such as temperature shift or chemical induction, and cells are cultivated for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known by the skilled artisan.

IV. Transgenic animals

The present invention also provides methods and compositions for the generation of non-human animals and plants that express recombinant CD164 polypeptides. The animals or plants can be transgenic, i.e. each of their cells contains a gene encoding the CD164 polypeptide, or, alternatively, a polynucleotide encoding the polypeptide can be introduced into somatic cells of the animal or plant, e.g. into

mammary secretory epithelial cells of a mammal. In preferred embodiments, the non-human animal is a mammal such as a cow, sheep, goat, pig, or rabbit.

Methods of making transgenic animals such as mammals are well known to those of skill in the art, and any such method can be used in the present invention. Briefly, transgenic mammals can be produced, e.g., by transfecting a pluripotential stem cell such as an ES cell with a polynucleotide encoding a polypeptide of interest. Successfully transformed ES cells can then be introduced into an early stage embryo that is then implanted into the uterus of a mammal of the same species. In certain cases, the transformed ("transgenic") cells will comprise part of the germ line of the resulting animal, and adult animals comprising the transgenic cells in the germ line can then be mated to other animals, thereby eventually producing a population of transgenic animals that have the transgene in each of their cells, and which can stably transmit the transgene to each of their offspring. Other methods of introducing the polynucleotide can be used, for example introducing the polynucleotide encoding the polypeptide of interest into a fertilized egg or early stage embryo via microinjection. Alternatively, the transgene may be introduced into an animal by infection of zygotes with a retrovirus containing the transgene (Jaenisch, R. (1976) Proc Natl Acad Sci USA 73, 1260-1264). Methods of making transgenic mammals are described, e.g., in Wall et al. (1992) J Cell Biochem 1992 49(2): 113-20; Hogan, et al. (1986) in Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; in WO 91/08216; or in US Patent Number 4,736,866.

In a preferred method, the polynucleotides ares microinjected into the fertilized oocyte. Typically, fertilized oocytes are microinjected using standard techniques, and then cultured in vitro until a "pre-implantation embryo" is obtained. Such pre-implantation embryos preferably contain approximately 16 to 150 cells. Methods for culturing fertilized oocytes to the pre-implantation stage are described, e.g., by Gordon et al. ((1984) Methods in Enzymology, 101, 414); Hogan et al. ((1986) in Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y) (for the mouse embryo); Hammer et al. ((1985) Nature, 315, 680) (for rabbit and porcine embryos); Gandolfi et al. ((1987) J Reprod Fert 81, 23-28); Rexroad et al. ((1988) J Anim Sci 66, 947-953) (for ovine embryos); and Eyestone et al. ((1989) J Reprod Fert 85, 715-720); Camous et al. ((1984) J Reprod Fert 72, 779-785); and Heyman et al. ((1987) Theriogenology 27, 5968) (for bovine embryos); the disclosures of each of which are incorporated herein in their entireties. Pre-implantation embryos are then transferred to an appropriate

female by standard methods to permit the birth of a transgenic or chimeric animal, depending upon the stage of development when the transgene is introduced.

As the frequency of transgene incorporation is often low, the detection of transgene integration in pre-implantation embryos is often desirable using any of the herein-described methods. Any of a number of methods can be used to detect the presence of a transgene in a pre-implantation embryo. For example, one or more cells may be removed from the pre-implantation embryo, and the presence or absence of the transgene in the removed cell or cells can be detected using any standard method e.g. PCR. Alternatively, the presence of a transgene can be detected in utero or post partum using standard methods.

In a particularly preferred embodiment of the present invention, transgenic mammals are generated that secrete recombinant CD164 polypeptides in their milk. As the mammary gland is a highly efficient protein-producing organ, such methods can be used to produce protein concentrations in the gram per liter range, and often significantly more. Preferably, expression in the mammary gland is accomplished by operably linking the polynucleotide encoding the CD164 polypeptide to a mammary gland specific promoter and, optionally, other regulatory elements. Suitable promoters and other elements include, but are not limited to, those derived from mammalian short and long WAP, alpha, beta, and kappa, casein, alpha and beta lactoglobulin, beta-CN 5' genes, as well as the the mouse mammary tumor virus (MMTV) promoter. Such promoters and other elements may be derived from any mammal, including, but not limited to, cows, goats, sheep, pigs, mice, rabbits, and guinea pigs. Promoter and other regulatory sequences, vectors, and other relevant teachings are provided, e.g., by Clark (1998) J Mammary Gland Biol Neoplasia 3:337-50; Jost et al. (1999) Nat Biotechnol 17:160-4; U.S. Patent Nos. 5,994,616; 6,140,552; 6,013,857; Sohn et al. (1999) DNA Cell Biol. 18:845-52; Kim et al. (1999) J Biochem (Japan) 126:320-5; Soulier et al. (1999) Euro J Biochem 260:533-9; Zhang et al. (1997) Chin J Biotech 13:271-6; Rijnkels et al. (1998) Transgen Res 7:5-14; Korhonen et al. (1997) Euro J Biochem 245:482-9; Uusi-Oukari et al. (1997) Transgen Res 6:75-84; Hitchin et al. (1996) Prot Expr Purif 7:247-52; Platenburg et al. (1994) Transgen Res 3:99-108; Heng-Cherl et al. (1993) Animal Biotech 4:89-107; and Christa et al. (2000) Euro J Biochem 267:1665-71; the entire disclosures of each of which is herein incorporated by reference.

In another embodiment, the polypeptides of the invention can be produced in milk by introducing polynucleotides encoding the polypeptides into somatic cells of the mammary gland in vivo, e.g. mammary secreting epithelial cells. For example, plasmid DNA can be infused through the nipple canal, e.g. in association with DEAE-

dextran (see, e.g., Hens et al. (2000) Biochim. Biophys. Acta 1523:161-171), in association with a ligand that can lead to receptor-mediated endocytosis of the construct (see, e.g., Sobolev et al. (1998) 273:7928-33), or in a viral vector such as a retroviral vector, e.g. the Gibbon ape leukemia virus (see, e.g., Archer et al. (1994) PNAS 91:6840-6844). In any of these embodiments, the polynucleotide may be operably linked to a mammary gland specific promoter, as described above, or, alternatively, any strongly expressing promoter such as CMV or MoMLV LTR.

The suitability of any vector, promoter, regulatory element, etc. for use in the present invention can be assessed beforehand by transfecting cells such as mammary epithelial cells, e.g. MacT cells (bovine mammary epithelial cells) or GME cells (goat mammary epithelial cells), in vitro and assessing the efficiency of transfection and expression of the transgene in the cells.

For *in vivo* administration, the polynucleotides can be administered in any suitable formulation, at any of a range of concentrations (e.g. 1-500 µg/ml, preferably 50-100 µg/ml), at any volume (e.g. 1-100 ml, preferably 1 to 20 ml), and can be administered any number of times (e.g. 1, 2, 3, 5, or 10 times), at any frequency (e.g. every 1, 2, 3, 5, 10, or any number of days). Suitable concentrations, frequencies, modes of administration, etc. will depend upon the particular polynucleotide, vector, animal, etc., and can readily be determined by one of skill in the art.

In a preferred embodiment, a retroviral vector such as Gibbon ape leukemia viral vector is used, as described in Archer et al. ((1994) PNAS 91:6840-6844). As retroviral infection typically requires cell division, cell division in the mammary glands can be stimulated in conjunction with the administration of the vector, e.g. using a factor such as estrodiol benzoate, progesterone, reserpine, or dexamethasone. Further, retroviral and other methods of infection can be facilitated using accessory compounds such as polybrene.

In any of the herein-described methods for obtaining CD164 polypeptides from milk, the quantity of milk obtained, and thus the quantity of CD164 polypeptides produced, can be enhanced using any standard method of lacation induction, e.g. using hexestrol, estrogen, and/or progesterone.

Typically, the encoded polypeptide will include a signal sequence to ensure the secretion of the protein into the milk. Where a full-length CD164 sequence is used, the full-length protein can, e.g., be isolated from milk and cleaved *in vitro* using a suitable protease. Alternatively, a second, protease-encoding polynucleotide can be introduced into the animal or into the mammary gland cells, whereby expression of the protease results in the cleavage of the CD164 polypeptide *in vivo*, thereby allowing the direct isolation of e.g. soluble CD164 polypeptide fragments from milk.

V. Pharmaceutical or Physiologically Acceptable Compositions of the Invention

The soluble CD164 polypeptides of the invention can be administered to non-human animals and/or humans, alone or in pharmaceutical or physiologically acceptable compositions where they are mixed with suitable carriers or excipient(s). The pharmaceutical or physiologically acceptable composition is then provided at a therapeutically effective dose. A therapeutically effective dose refers to that amount of soluble CD164 sufficient to result in prevention or amelioration of symptoms or physiological status of inflammation or/and autoimmune diseases or disorders as determined by the methods described herein.

Further preferred embodiments relate to methods for the prophylaxis or treatment of inflammation or/and autoimmune disorders, such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis comprising administering to a subject in need of treatment (alternatively on a timed daily basis) soluble CD164 polypeptides (or polynucleotide encoding said polypeptide) in dosage amount and for a period sufficient to reduce cytokines levels in said animal or human subject.

Routes of Administration.

Suitable routes of administration include oral, nasal, rectal, transmucosal, or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intrapulmonary (inhaled) or intraocular injections using methods known in the art. Other particularly preferred routes of administration are aerosol and depot formulation. Sustained release formulations, particularly depot, of the invented medicaments are expressly contemplated.

For parenteral (e.g. intravenous, subcutaneous, intramuscular) administration, the active protein(s) can be formulated as a solution, suspension, emulsion or lyophilised powder in association with a pharmaceutically acceptable parenteral vehicle (e.g. water, saline, dextrose solution) and additives that maintain isotonicity (e.g. mannitol) or chemical stability (e.g. preservatives and buffers). The formulation is sterilized by commonly used techniques.

The active ingredients of the pharmaceutical composition according to the invention can be administered to an individual in a variety of ways. The routes of administration include intradermal, transdermal (e.g. in slow release formulations), intramuscular, intraperitoneal, intravenous, subcutaneous, oral, epidural, topical, and intranasal routes. Any other therapeutically efficacious route of administration can be used, for example absorption through epithelial or endothelial tissues or by gene therapy wherein a DNA molecule encoding the active agent is administered to the patient (e.g. via a vector), which causes the active agent to be expressed and secreted in vivo. In addition, the protein(s) according to the invention can be administered together with other components of biologically active agents such as pharmaceutically acceptable surfactants, excipients, carriers, diluents and vehicles.

Composition/Formulation

Pharmaceutical or physiologically acceptable compositions and medicaments for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries. Proper formulation is dependent upon the route of administration chosen.

Certain of the medicaments described herein will include a pharmaceutically or physiologically acceptable carrier and at least one polypeptide that is a soluble CD164 polypeptide of the invention. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer such as a phosphate or bicarbonate buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Pharmaceutical or physiologically acceptable preparations that can be taken orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable gaseous propellant, *e.g.*, carbon dioxide. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin, for use in an inhaler or insufflator, may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical or physiologically acceptable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Aqueous suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder or lyophilized form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical or physiologically acceptable compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Depending on the mode of administration, the compounds of the invention can be formulated with the appropriate diluents and carriers to form ointments, creams, foams, and solutions having from about 0.01% to about 15% by weight, preferably from about 1% to about 10% by weight of the compounds.

The term "pharmaceutically acceptable" is meant to encompass any carrier, which does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered. For example, for parenteral administration, the active protein(s) may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

Effective Dosage.

Pharmaceutical or physiologically acceptable compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve their intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes or encompasses a concentration point or range shown to decrease cytokine expression in an *in vitro* system. Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50, (the dose lethal to 50% of the test population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD5O and ED5O. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50, with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (*See, e.g.,* Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1).

Dosage intervals can also be determined using the value for the minimum effective concentration. Compounds should be administered using a regimen that maintains plasma levels above the minimum effective concentration for 10-90% of the time, preferably between 30-90%; and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

A preferred dosage range for the amount of a soluble CD164 polypeptide of the invention, which can be administered on a daily or regular basis to achieve desired results, including a reduction in levels of cytokines. A typical dosage may range from about 0.1 mg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 mg/kg up to about 1 mg/kg; or from 1mg/kg up to about 5 mg/kg, or from 5 mg/kg up to about 100 mg/kg. Of course, these daily dosages can be delivered or administered in small amounts periodically during the course of a day. It is noted that these dosage ranges are only preferred ranges and are not meant to be limiting to the invention.

The bioavailability of the active protein(s) according to the invention can also be ameliorated by using conjugation procedures which increase the half-life of the molecule in the human body, for example linking the molecule to polyethylenglycol, as described in the PCT Patent Application WO 92/13095.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

The substances of the invention may be administered daily or every other day, of less frequent. Preferably, one or more of the substances of the invention are administered one, twice or three times per week.

The daily doses are usually given in divided doses or in sustained release form effective to obtain the desired results. Second or subsequent administrations can be performed at a dosage which is the same, less than or greater than the initial or previous dose administered to the individual. A second or subsequent administration can be administered during or prior to onset of the disease.

According to the invention, the substances of the invention can be administered prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions.

VI Methods of Treatment

Treatment of mice with CD164 polypeptides result in reduction of excess inflammatory and/or autoimmune response, thus serving as a viable treatment for inflammatory or/and autoimmune disorders, including, but not limited to, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, as well as other inflammatory disorders such as other rheumatic diseases, juvenile idiopathic arthritis, psoriatic arthritis, osteoarthritis, spondylarthropathies, ulcerative colitis, inflammatory bowel disease, endotoxemia, Crohn's disease, Still's disease, uveitis, Wegener's granulomatosis, Behcet's disease, scleroderma, Sjogren's syndrome, sarcoidosis, pyodema gangrenosum and polymyositis/dermatomyositis, myocarditis, psoriasis, hepatitis C, allergies, allergic inflammation, allergic airway inflammation, allergic asthma, bronchial asthma, mesenteric infarction, stroke, fibrosis and tuberculosis.

Treatment comprises providing pharmaceutically acceptable soluble CD164 polypeptides to an individual. The exact amount of soluble CD164 provided would be determined through clinical trials under the guidance of qualified physicians, but would be expected to be in the range of 5-7 mg per individual per day. In general, a preferred range would be from 0.5 to 14 mg per individual per day, with a highly preferred range being between 1 and 10 mg per individual per day.

In further embodiments, soluble CD164 polypeptides of the present invention are administered to individuals, preferably individuals susceptible to inflammation or/and autoimmune disorders or those who suffer from inflammation or/and

autoimmune disorders, whose levels of CD164 are at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, about 100% or 100% lower than healthy individuals, preferably healthy individuals as determined by a physician using normal standards in the art. Methods to determine and compare the levels of CD164 in individuals are well-known in the art and include, but are not limited to using an antibody specific for CD164 in a format such as a Radio Immune Assay, ELISA, Western blot, dotblot, or as part of an array, for example.

Having now described the invention, it will be more readily understood by reference to the following examples that are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

Example 1: Cloning of sf-CD164

The original cDNA encoding CD164 antigen (or putative mucin core protein 24) was purchased from Invitrogen (Invitrogen clone ID: CS0DI082DC08) and the sequence encoding amino acids 1-163 (extracellular domain) was submitted to Invitrogen for subcloning to generate plasmid 12843 using their Gateway™ cloning technology.

Example 2: Throughput expression in mammalian cells and purification of the cloned, His-tagged sf-CD164.

Human Embryonic Kidney 293 cells expressing the Epstein-Barr virus Nuclear Antigen (HEK293-EBNA, Invitrogen) were maintained in suspension in Ex-cell VPRO serum-free medium (seed stock, maintenance medium, JRH). Sixteen to 20 hours prior to transfection (Day-1), cells were seeded in 2x T225 flasks (50 ml per flask in DMEM / F12 (1:1) containing 2% FBS seeding medium (JRH) at a density of $2x10^5$ cells/ ml). The next day (transfection day0) the transfection took place by using the JetPEI™ reagent (2µl/µg of plasmid DNA, PolyPlus-transfection). For each flask, 113 µg of cDNA (number #13498) was co-transfected with 2.3 µg of GFP (fluorescent reporter gene). The transfection mix was then added to the 2xT225 flasks and incubated at 37°C (5%CO_2) for 6 days. Confirmation of positive transfection was done by qualitative fluorescence examination at day 1 and day 6 (Axiovert 10 Zeiss). On day 6 (harvest day), supernatants (100ml) from the two flasks were pooled and centrifuged (4°C, 400g) and placed into a pot bearing a unique identifier.

One aliquot (500ul) was kept for QC of the 6His-tagged protein (internal bioprocessing QC). The corresponding delivery sheets can be found in L.Rey's notebook number 11006 pages 54 (batch 1 from T Flasks) and Battle's notebook11140 p32 for intermediate 500ml scale-ups.

Purification process

The 100 and 500 ml culture medium samples containing the recombinant protein with a C-terminal 6His tag were diluted with one volume cold buffer A (50 mM NaH_2PO_4; 600 mM NaCl; 8.7 % (w/v) glycerol, pH 7.5) to final volumes of 200 and 1000 ml, respectively. The samples were filtered through a 0.22 µm sterile filter (Millipore, 500 ml filter unit) and kept at 4ºC in sterile square media bottle (Nalgene).

The purification was performed at 4ºC on the VISION workstation (Applied Biosystems) connected to an automatic sample loader (Labomatic). The purification procedure was composed of two sequential steps, metal affinity chromatography on

a Poros 20 MC (Applied Biosystems) column charged with Ni ions (4.6 x 50 mm, 0.83 ml), followed by gel filtration on a Sephadex G-25 medium (Amersham Pharmacia) column (1,0 x 10 cm).

For the first chromatography step the metal affinity column was regenerated with 30 column volumes of EDTA solution (100 mM EDTA; 1 M NaCl; pH 8.0), recharged with Ni ions through washing with 15 column volumes of a 100 mM $NiSO_4$ solution, washed with 10 column volumes of buffer A, followed by 7 column volumes of buffer B (50 mM NaH_2PO_4; 600 mM NaCl; 8.7 % (w/v) glycerol, 400 mM; imidazole, pH 7.5), and finally equilibrated with 15 column volumes of buffer A containing 15 mM imidazole. The sample was transferred, by the Labomatic sample loader, into a 200 ml sample loop and subsequently charged onto the Ni metal affinity column at a flow rate of 10 ml/min. For the 1000 ml sample the charging procedure was repeated 5 times. The column was washed with 12 column volumes of buffer A, followed by 28 column volumes of buffer A containing 20 mM imidazole. During the 20 mM imidazole wash loosely attached contaminating proteins were elution of the column. The recombinant His-tagged protein was finally eluted with 10 column volumes of buffer B at a flow rate of 2 ml/min, and the eluted protein was collected in a 1.6 ml fraction.

For the second chromatography step, the Sephadex G-25 gel-filtration column was regenerated with 2 ml of buffer D (1.137 M NaCl; 2.7 mM KCl; 1.5 mM KH_2PO_4; 8 mM Na_2HPO_4; pH 7.2), and subsequently equilibrated with 4 column volumes of buffer C (137 mM NaCl; 2.7 mM KCl; 1.5 mM KH_2PO_4; 8 mM Na_2HPO_4; 20 % (w/v) glycerol; pH 7.4). The peak fraction eluted from the Ni-column was automatically, through the integrated sample loader on the VISION, loaded onto the Sephadex G-25 column and the protein was eluted with buffer C at a flow rate of 2 ml/min. The desalted sample was recovered in a 2.2 ml fraction. The fraction was filtered through a 0.22 µm sterile centrifugation filter (Millipore), aliquoted, frozen and stored at –80C. An aliquot of the sample was analyzed on SDS-PAGE (4-12 % NuPAGE gel; Novex) by coomassie staining and Western blot with anti-His antibodies.

Coomassie staining. The NuPAGE gel was stained in a 0.1 % coomassie blue R250 staining solution (30 % methanol, 10 % acetic acid) at room temperature for 1 h and subsequently destained in 20 % methanol, 7.5 % acetic acid until the background was clear and the protein bands clearly visible.

Western blot. Following the electrophoresis the proteins were electrotransferred from the gel to a nitrocellulose membrane at 290 mA for 1 hour at 4ºC. The membrane was blocked with 5 % milk powder in buffer E (137 mM NaCl;

2.7 mM KCl; 1.5 mM KH_2PO_4; 8 mM Na_2HPO_4; 0.1 % Tween 20, pH 7.4) for 1 h at room temperature, and subsequently incubated with a mixture of 2 rabbit polyclonal anti-His antibodies (G-18 and H-15, 0.2ug/ml each; Santa Cruz) in 2.5 % milk powder in buffer E overnight at 4°C. After further 1 hour incubation at room temperature, the membrane was washed with buffer E (3 x 10 min), and then incubated with a secondary HRP-conjugated anti-rabbit antibody (DAKO, HRP 0399) diluted 1/3000 in buffer E containing 2.5 % milk powder for 2 hours at room temperature. After washing with buffer E (3 x 10 minutes), the membrane was developed with the ECL kit (Amersham Pharmacia) for 1 min. The membrane was subsequently exposed to a Hyperfilm (Amersham Pharmacia), the film developed and the western blot image visually analyzed.

Protein assay. The protein concentration was determined using the BCA protein assay kit (Pierce) with bovine serum albumin as standard.

Example 3: Cytokine expression modulation assays

3.1 Introduction: The following *in vitro* cell-based tri-replicas assays measure the effects of sf-CD164 on cytokine secretion induced by Concanavalin A (Con A) acting on different human peripheral blood mononuclear cells (hPBMC) cells as measured by a cytokine bead array (CBA) assay for IL-2, IFN-γ, TNF-α, IL-5, IL-4 and IL-10.

The optimal conditions are 100 000 cells/well in 96-well plates and 100µl final in 2 % glycerol.

The optimal concentration of mitogen (ConA) is 5 ng/ml.

The optimal time for the assay is 48 h.

The read-out choice is the CBA.

3.2 Equipments and softwares

- 96 well microtiter plate photometer EX (Labsystem).
- Graph Pad Prism Software
- Excel software
- Flow cytometer Becton-Dickinson
- CBA Analysis software
- Hood for cell culture
- Incubator for cell culture
- Centrifuge

- Pipettes

3.3 Materials and Reagents

- Buffy coat
- DMEM GIBCO
- Human serum type AB SIGMA
- L-Glutamine GIBCO
- Penicillin-Streptomycin GIBCO
- Ficoll PHARMACIA
- 96 well microtiter plate for cell culture COSTAR
- Concanavalin A SIGMA
- Human Th1/Th2 Cytokine CBA Kit Becton-Dickinson
- PBS GIBCO
- FALCON 50 ml sterile Becton-Dickinson
- BSA SIGMA
- Glycerol MERCK
- DMSO SIGMA
- 96 well microtiter plate conical bottom NUNC

3.4 METHOD

3.4.1 Purification of Human PBMC from a buffy coat

The buffy coat 1 to 2 was diluted with DMEM. 25 ml of diluted blood was thereafter slowly added onto a 15 ml layer of Ficoll in a 50 ml Falcon tube, and tubes were centrifuged (2000 rpm, 20 min, at RT without brake). The interphase (ring) was then collected and the cells were washed with 25 ml of DMEM followed by a centrifuge step (1200 rpm, 5 min). This procedure was repeated three times. A buffy coat gave approximately 600 x 10^6 total cells.

3.4.2 Screening

80 μl of 1.25 x 10^6 cells/ml were diluted in DMEM+2.5% Human Serum+1% L-Glutamine+1% Penicillin-Streptomycin and thereafter added to a 96 well microtiter plate.

10μl were added per well (one condition per well): Proteins were diluted in PBS+20%Glycerol (the final dilution of the proteins is 1/10).

10μl of the ConA Stimuli were then added per well (one condition per well):

- ConA 50μg/ml (the final concentration of ConA is 5μg/ml)

After 48 h, cell supernatants were collected and human cytokines were measured by Human Th1/Th2 Cytokine CBA Kit Becton-Dickinson.

3.4.3 CBA analysis

(for more details, refer to the booklet in the CBA kit)

i) Preparation of mixed Human Th1/Th2 Capture Beads

The number of assay tubes that were required for the experiment was determined. Each capture bead suspension was vigorously vortexed for a few seconds before mixing. For each assay to be analysed, 10µl aliquot of each capture bead were added into a single tube labelled "mixed capture beads". The Bead mixture was thoroughly vortexed.

ii) Preparation of test samples

Supernatants were diluted (1:4) using the Assay Diluent (20µl of supernatants + 60µl of Assay Diluent). The sample dilution was then mixed before transferring samples into a 96 wells microtiter plate conical bottom (Nunc).

iii) Human Th1/Th2 Cytokine CBA Assay Procedure

50µl of the diluted supernatants were added into a 96 wells microtiter plate conical bottom (Nunc). 50µl of the mixed capture beads were added followed by 50µl addition of the Human Th1/Th2 PE Detection Reagent. The plate was then incubated for 3 hours at RT and protected from direct exposure to light followed by centrifugation at 1500rpm for 5 minutes. The supernatant was then carefully discarded. In a subsequent step, 200µl of wash buffer were twice added to each well, centrifuged at 1500rpm for 5 minutes and supernatant carefully discarded. 130µl of wash buffer were thereafter added to each well to resuspend the bead pellet. The samples were finally analysed on a flow cytometer. The data were analysed using the CBA Application Software, Activity Base and Microsoft Excel software.

3.5 Results

For each cytokine, two tables are presented:

i) First table: First column indicates the concentration in µg/ml of sf-CD164, and columns two to five are replicas indicating the percentage of cytokine release.

ii) Second table: Values (in µg/ml) of line two correspond to EC50 (the concentration that inhibits cytokine release to 50%) for each replica (EXP1, EXP2 and EXP3). Mean EC50 and its standard deviation are also indicated.

3.5.1 IFN-γ

TABLE IV

CONCENTRATION μG/ML	%CYTOKINE RELEASE	% CYTOKINE RELEASE	% CYTOKINE RELEASE
6.200	8.76	33.18	26.65
3.100	16.98	38.70	40.11
1.550	42.08	48.31	56.58
0.775	64.85	54.09	58.14
0.388	103.90	70.29	67.19
0.194	94.05	69.66	82.71
0.097	89.89	70.29	81.97
0.048	129.08	69.03	93.01
0.024	130.25	72.22	93.01
0.012	124.49	82.69	97.30

TABLE V

EC50 EXP1	EC50 EXP2	EC50 EXP3
0.7726392	0.9175135	0.8290225
mean EC50	0.8397251	
SD	0.0730277	

3.5.2 TNF-α

TABLE VI

CONCENTRATION μG/ML	% CYTOKINE RELEASE	% CYTOKINE RELEASE	% CYTOKINE RELEASE
6.200	5.28	13.50	7.93
3.100	13.33	21.88	16.35
1.550	27.89	33.75	27.06
0.775	44.72	48.10	35.62
0.388	73.12	71.00	49.01
0.194	75.86	72.30	80.02
0.097	88.69	77.38	77.17
0.048	94.55	85.85	77.88
0.024	94.55	83.55	93.76

0.012	88.69	87.41	94.18

TABLE VII

EC50 EXP1	EC50 EXP2	EC50 EXP3
0.7197047	0.7899553	0.4656914
mean EC50	0.6584505	
SD	0.1705897	

3.5.3 IL-2

TABLE VIII

CONCENTRATION μG/ML	% CYTOKINE RELEASE	% CYTOKINE RELEASE	% CYTOKINE RELEASE
6.2	2.05	6.53	8.64
3.1	9.25	8.24	16.20
1.55	10.75	12.64	28.54
0.775	22.55	18.93	40.05
0.3875	54.22	34.22	57.19
0.19375	55.59	51.45	75.97
0.096875	81.06	58.67	79.47
0.0484375	81.80	57.11	95.59
0.02421875	86.52	56.84	92.21
0.012109375	77.70	63.37	96.02

TABLE IX

EC50 EXP1	EC50 EXP2	EC50 EXP3
0.4762997	0.3919559	0.5021433
mean EC50	0.4567996	
SD	0.0576238	

3.5.4 IL-4

TABLE X

CONCENTRATION μG/ML	% CYTOKINE RELEASE	% CYTOKINE RELEASE	% CYTOKINE RELEASE
6.200	1.98	11.41	22.85
3.100	8.58	19.02	35.75
1.550	13.86	24.54	51.34
0.775	26.18	44.70	60.48
0.388	49.50	58.39	57.80
0.194	56.99	75.70	97.58
0.097	104.07	73.42	106.18
0.048	69.31	84.07	97.58
0.024	97.47	80.08	85.75
0.012	104.07	84.83	106.18

TABLE XI

EC50 EXP1	EC50 EXP2	EC50 EXP3
0.3129018	0.5886195	0.6466119
mean EC50	0.5160444	
SD	0.1783002	

3.5.5 IL-5

TABLE XII

CONCENTRATION μG/ML	% CYTOKINE RELEASE	% CYTOKINE RELEASE	% CYTOKINE RELEASE
6.200	2.931596091	30.78900852	32.2595385
3.100	10.88633636	46.38705876	50.44551062
1.550	20.04114521	63.59205099	60.77221841
0.775	35.43631065	78.39734319	79.96344528
0.388	77.71301217	79.49006374	67.44345442
0.194	85.15343734	85.48931384	98.2408042
0.097	123.7956455	84.69655579	64.3363034

0.048	86.73067032	88.63892013	66.80374686
0.024	95.02828733	94.46676308	72.10418095
0.012	100.3771644	91.91708179	92.02650217

TABLE XIII

EC50 EXP1	EC50 EXP2	EC50 EXP3
0.6262594	1.7624317	#NoFit!
mean EC50	1.1943456	
SD	0.8033951	

3.5.6 IL-10

TABLE XIV

CONCENTRATION μG/ML	% CYTOKINE RELEASE	% CYTOKINE RELEASE	% CYTOKINE RELEASE
6.200	6.31	17.90	24.60
3.100	13.77	21.69	36.09
1.550	22.94	35.29	56.59
0.775	47.23	51.97	62.67
0.388	75.33	70.20	90.61
0.194	83.75	79.95	92.31
0.097	110.13	81.49	119.26
0.048	122.37	88.74	138.01
0.024	133.46	89.57	111.86
0.012	163.86	102.28	111.86

TABLE XV

EC50 EXP1	EC50 EXP2	EC50 EXP3
0.23954	0.5313631	0.8126636
mean EC50	0.5278556	

SD	0.2865779	

3.6 Conclusion

In vitro, sf-CD164 has a consistent inhibitory effect on all cytokines tested (IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10). Based on the EC50 value, best inhibition occurs for IL-2, followed by IL-4, IL-10, TNF-α, IFN-γ, and IL-5 respectively.

ADDITIONAL REFERENCES

1. Herrick C.A. and Bottomly K., "To respond or not to respond: T cells in allergic asthma.", Nature Reviews|Immunology: 2003 May; Volume 3.
2. O Gor D. et al., "Th1-Th2: a Procrustean paradigm.", Nature Immunology; 4(6)-June 2003: 503-505.
3. Tutuncu Z. et al., "Anti-TNF therapy for other inflammatory conditions.", Clin. Exp. Rheumatol. 2002 Nov-Dec; 20(6 Suppl 28): S146-51.

CLAIMS

1. Use of a soluble CD164, or an isoform, mutein, fused protein, functional derivative, fragment, active fraction or salt thereof, for the manufacture of a medicament for treatment and/or prevention of inflammation or/and autoimmune disorders.

2. Use of a soluble fragment of CD164 as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 2, or an isoform, mutein, fused protein, functional derivative, active fraction or salt thereof, for the manufacture of a medicament for treatment and/or prevention of inflammation or/and autoimmune disorders.

3. The use according to any of the preceding claims, wherein said soluble CD164 is

(a) the polypeptide as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6;

(b) a polypeptide exhibiting at least about 85% identity to the polypeptide as set forth in any of SEQ ID NO:1, or SEQ ID NO: 6;

(c) a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO: 1, or SEQ ID NO: 6;

(d) a polypeptide comprising the extracellular part of an anchored CD164 protein;

(e) the mature form of the polypeptide whose sequence is recited in SEQ ID NO: 6;

(f) the histidine tag form of the polypeptides whose sequences are recited in SEQ ID NO: 1 (SEQ ID NO: 2) or SEQ ID NO: 6;

4. The use according to any of the preceding claims, wherein said soluble CD164 is a glycosylated CD164.

5. The use according to claim 4, wherein said glycosylated CD164 is glycosylated at any of the positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6.

6. The use according to any of the preceding claims, wherein said soluble CD164 is a phosphorylated CD164.

7. The use according to claim 6, wherein said phosphorylated CD164 is phosphorylated at any of the positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6.

8. The use according to any of the preceding claims, wherein said soluble CD164 is a myristoylated CD164.

9. The use according to claim 8, wherein said myristoylated CD164 is myristoylated at any of the positions as set forth in any of SEQ ID NO: 1 or SEQ ID NO: 6.

10. The use according to any of the preceding claims, wherein said fused protein comprises an Ig fusion.

11. A method of inhibiting cytokine expression in an individual comprising administering to said individual a composition comprising a soluble CD164.

12. The method according to claim 11, wherein said cytokine is TNF-α, or IFN-γ, or IL-2, or IL-4, or IL-5 or IL-10.

13. A pharmaceutical composition comprising a soluble CD164, in the presence of one or more pharmaceutically acceptable excipients, for the treatment of inflammation and/or autoimmune disorders.

ABSTRACT

The present invention relates to the field of inflammation and autoimmune disorders, in particular to the discovery of a soluble glycosylated CD164 fragment useful for reducing inflammation and for treating inflammatory and/or autoimmune disorders.

Figure 1. Amino acid alignment of soluble CD164 extracellular fragment (sf-CD164), delta 4-CD164, NP_006007 (Full length), MGC-24 and delta5-CD164. Signal peptide and extracellular domain are shown in dark gray and in light gray respectively.

```
FRAGMENT        ----------------------DKNTTQHPNVTTLAPISNVTSAPVTSLPLVTTPAPET
DELTA4CD164     MSRLSRSLLWAATCLGVLCVLSADKNTTQHPNVTTLAPISNVTSAPVTSLPLVTTPAPET
NP_006007       MSRLSRSLLWAATCLGVLCVLSADKNTTQHPNVTTLAPISNVTSAPVTSLPLVTTPAPET
MG24            MSRLSRSLLWAATCLGVLCVLSADKNTTQHPNVTTLAPISNVTSAPVTSLPLVTTPAPET
DELTA5CD164     MSRLSRSLLWAATCLGVLCVLSADKNTTQHPNVTTLAPISNVTSAPVTSLPLVTTPAPET
                                      ************************************

FRAGMENT        CEGRNSCVSCFNVSVVNTTCFWIECKDESYCSHNSTVSDCQVGNTTDFCSVSTATPVPTA
DELTA4CD164     CEGRNSCVSCFNVSVVNTTCFWIECKDESYCSHNSTVSDCQVGNTTDFCSAKPTVQP---
NP_006007       CEGRNSCVSCFNVSVVNTTCFWIECKDESYCSHNSTVSDCQVGNTTDFCSVSTATPVPTA
MG24            CEGRNSCVSCFNVSVVNTTCFWIECKDESYCSHNSTVSDCQVGNTTDFCSVSTATPVPTA
DELTA5CD164     CEGRNSCVSCFNVSVVNTTCFWIECKDESYCSHNSTVSDCQVGNTTDFCSVSTATP----
                ************************************************    .

FRAGMENT        NSTAKPTVQPSPSTTSKTVTTSGTTNNTVTPTSQPVRKSTFDA------------------
DELTA4CD164     ----------SPSTTSKTVTTSGTTNNTVTPTSQPVRKSTFDAASFIGGIVLVLGVQAVI
NP_006007       NSTAKPTVQPSPSTTSKTVTTSGTTNNTVTPTSQPVRKSTFDAASFIGGIVLVLGVQAVI
MG24            NSTAKPTVQPSPSTTSKTVTTSGTTNNTVTPTSQPVRKSTFDAASFIGGIVLVLGVQAVI
DELTA5CD164     ----------------VPTANSTGTTNNTVTPTSQPVRKSTFDAASFIGGIVLVLGVQAVI
                              * ...**********************

FRAGMENT        ------------------
DELTA4CD164     FFLYKFCKSKERNYHTL
NP_006007       FFLYKFCKSKERNYHTL
MG24            RNYIPDLKK---------
DELTA5CD164     FFLYKFCKSKERNYHTL
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Figure 2. SMART Domains alignment of soluble CD164 extracellular fragment, delta 4-CD164, NP_006007 (Full length), MGC-24 and delta5-CD164.

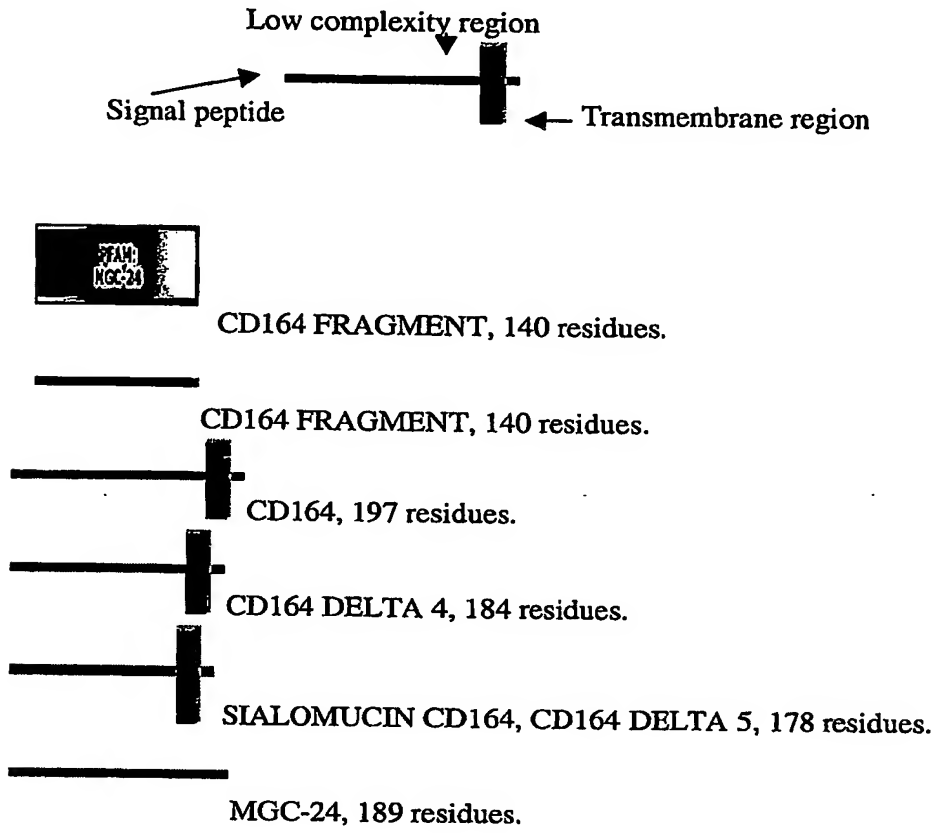


Figure 3. IFN-γ modulation

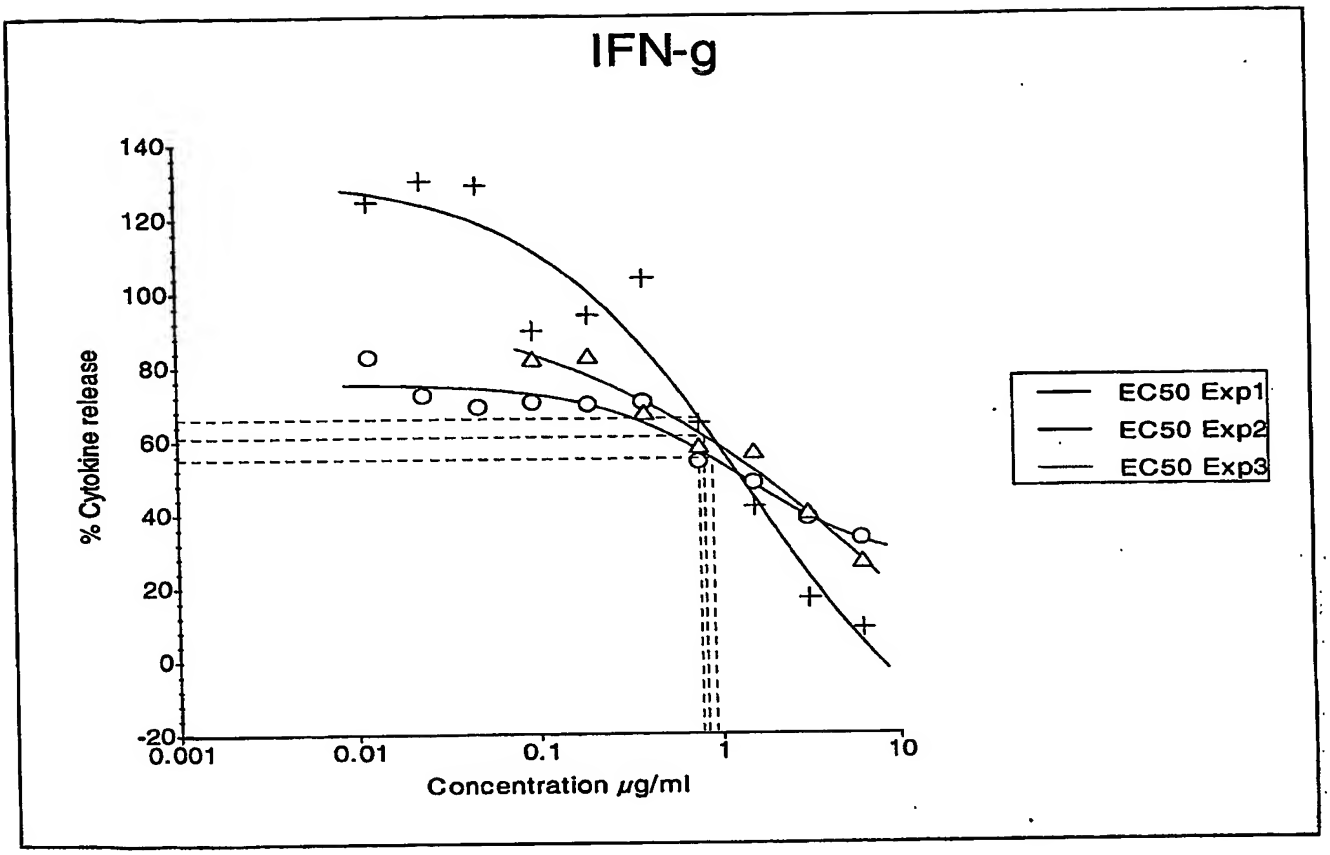


Figure 4. TNF-α modulation

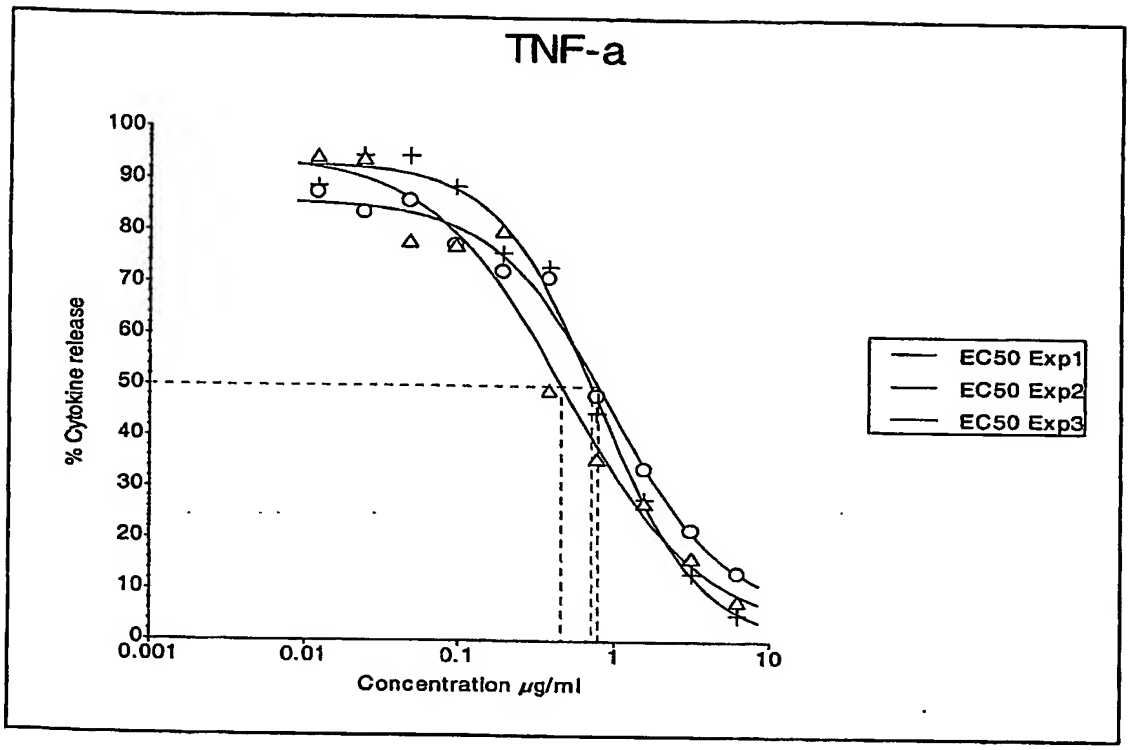


Figure 5. IL-2 modulation

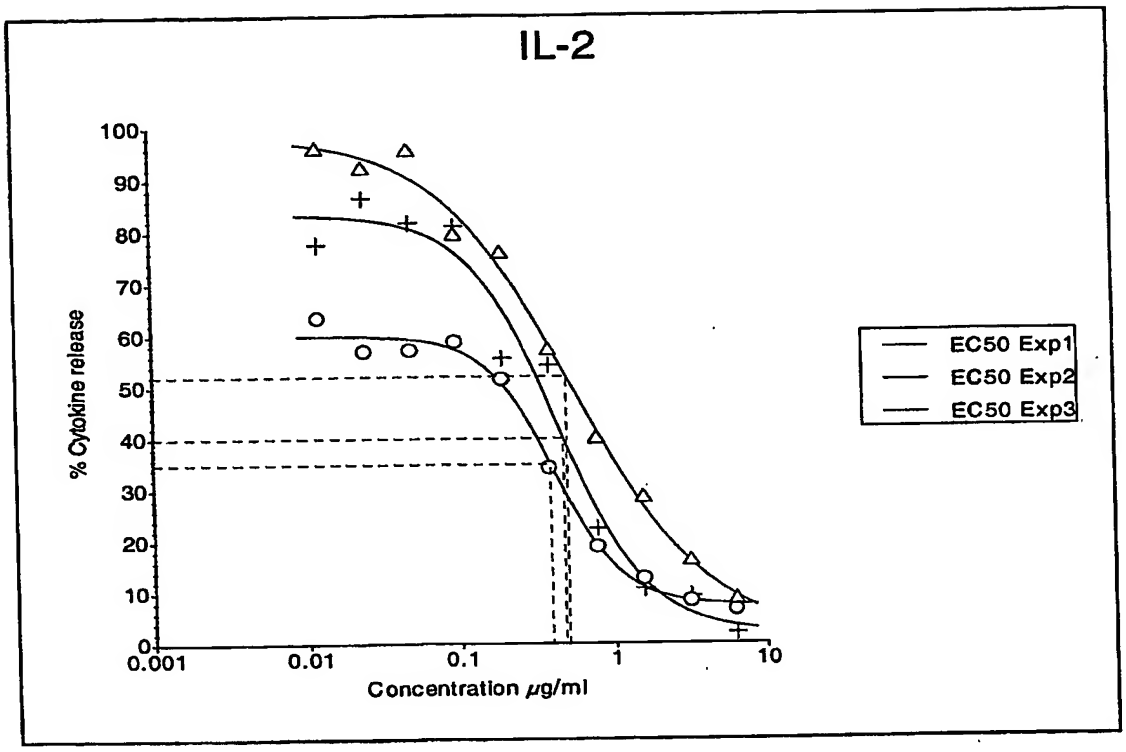


Figure 6. IL-4 modulation

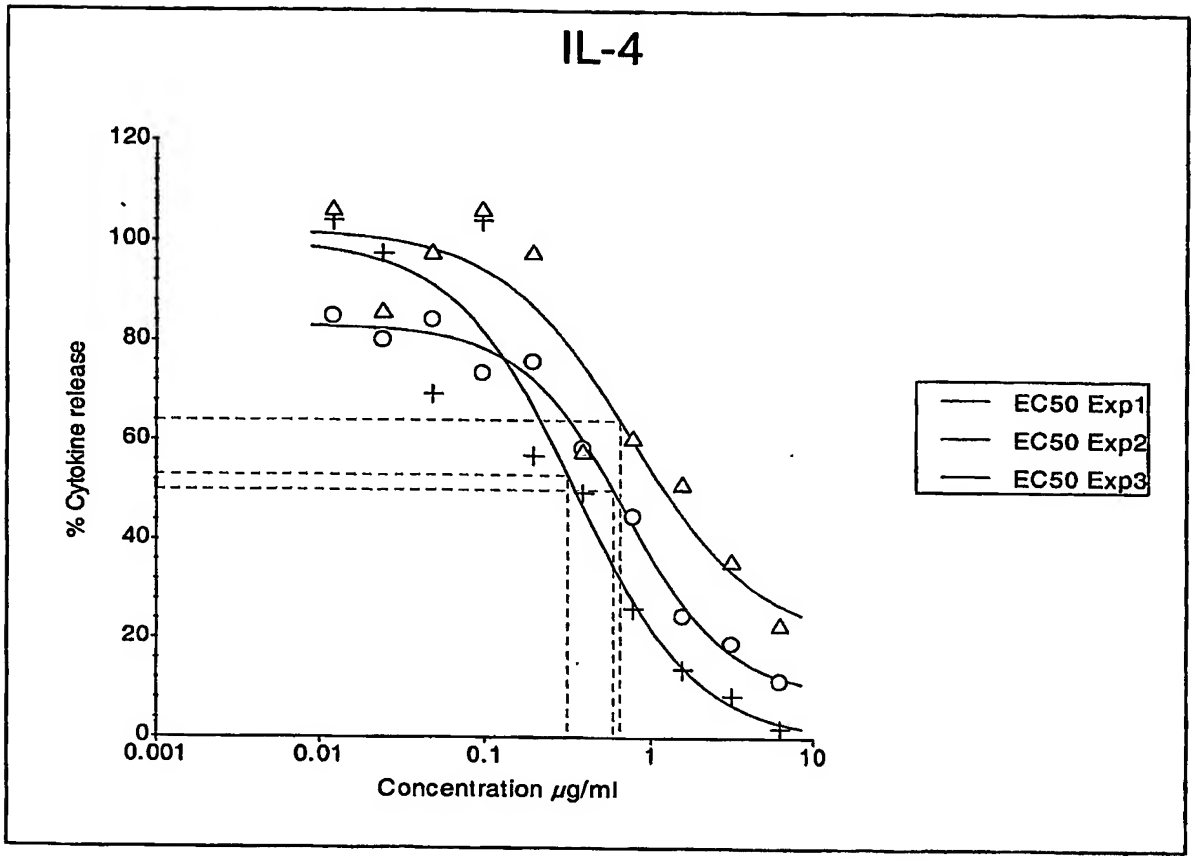


Figure 7. IL-5 modulation

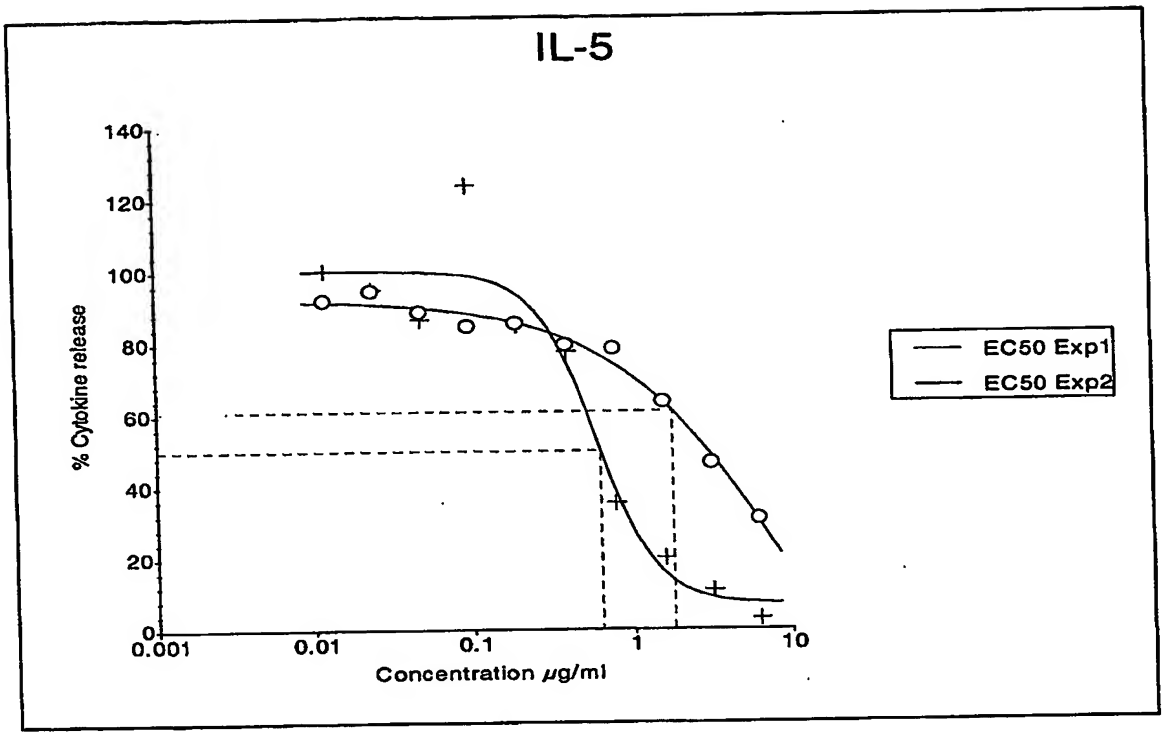
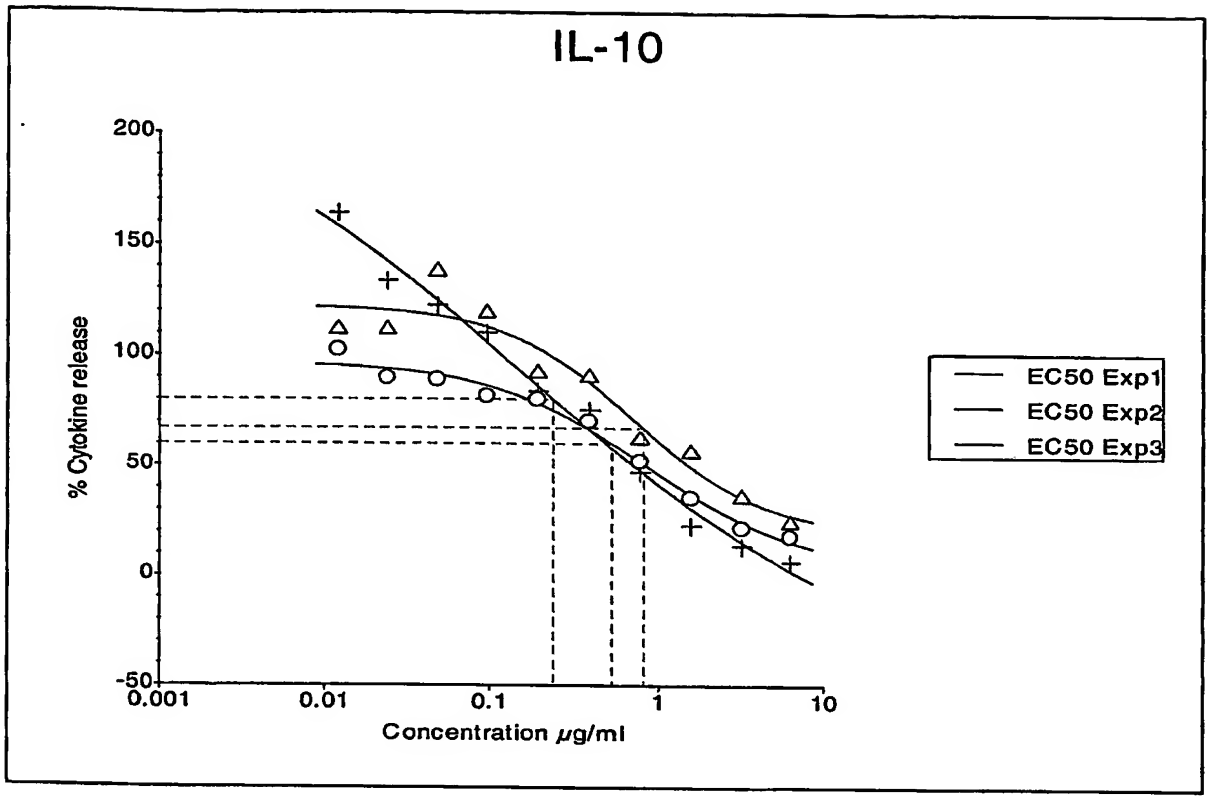


Figure 8. IL-10 modulation



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